Toxicity of the Organophosphate Chemical Warfare Agents GA, GB, and VX: Implications for Public Protection

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The nerve agents, GA, GB, and VX are organophosphorus esters that form a major portion of the total agent volume contained in the U.S. stockpile of unitary chemical munitions. Congress has mandated the destruction of these agents, which is currently slated for completion in 2004. The acute, chronic, and delayed toxicity of these agents is reviewed in this analysis. The largely negative results from studies of genotoxicity, carcinogenicity, developmental, and reproductive toxicity are also presented.

Nerve agents show few or delayed effects. At supralethal doses, GB can cause delayed neuropathy in antidote-protected chickens, but there is no evidence that it causes this syndrome in humans at any dose. Agent VX shows no potential for inducing delayed neuropathy in any species. In view of their lack of genotoxcity, the nerve agents are not likely to be carcinogens. The overreaching concern with regard to nerve agent exposure is the extraordinarily high acute toxicity of these substances. Furthermore, acute effects of moderate exposure such as nausea, diarrhea, inability to perform simple mental tasks, and respiratory effects may render the public unable to respond adequately to emergency instructions in the unlikely event of agent releaase, making early warning and exposure avoidance important. Likewise, exposure or self-contamination of first responders and medical personnel must be avoided. Control limits for exposure via surface contact of drinking water are needed, as are detection methods for low levels in water or foodstuffs. *Key words:* anticholinesterase, Chemical Stockpile Disposal Program, chemical warfare agent, GA, GB, nerve agent, organophosphate, organophosphorus-induced delayed neuropathy, sarin, tabun, VX. *Environ Health Perspect* 102:18–38(1994)

Recent events in the Middle East focused attention on the renewed threat of chemical warfare. The relative ease of warfare agent production from readily available industrial chemicals, the documented use of chemical weapons by Iraq against Kurdish civilians and Iranian military personnel (*1–3*), and the widespread possession of such weapons, raises the issue of chemical warfare proliferation to other conflicts (*4*) or to terrorist activity. Domestic attention to chemical munitions has also been sparked by the congressional mandate to destroy the U.S. unitary stockpile via incineration (PL 99-145 and PL 100-456); congressional directives to examine safe disposal of nonstockpile chemical material thought to be present in 32 states, the District of Columbia, and the Virgin Islands (PL 102-484; *5*); and the January 1993 signing of the Chemical Weapons Convention banning the manufacture, use, stockpiling, and transfer of chemical weapons (*6*).

The Chemical Stockpile Disposal Program (CSDP) of the U. S. Army will carry out the intent of Congress regarding the unitary stockpile; details are provided in the CSDP-Final Programmatic Environmental Impact Statement (FPEIS), (*7*) and summarized by Carnes (*8*) and Carnes and Watson (*9*). Workplans, budgets, and decision criteria for nonstockpile agents and munitions are currently under development (*5*).

Two nerve agents [GB (sarin), methylphosphonofluoridate isopropyl ester and VX, *S*-(diisopropylaminoethyl) methylphosphonothiolate *o*-ethyl ester], are stockpiled at six continental U. S. military installations. These installations include Anniston Army Depot (ANAD), near Anniston, Alabama; Blue Grass Army Depot (BGAD), near Richmond, Kentucky; Newport Army Ammunition Plant (NAAP), near Newport, Indiana; Pine Bluff Arsenal (PBA), near Pine Bluff, Arkansas; Tooele Army Depot (TEAD), near Tooele, Utah; and Umatilla Depot Activity (UMDA), near Umatilla, Oregon (see Table 1 for location of individual agent stockpiles) (*7*). A third nerve agent, GA (tabun; *N,N*-dimethyl phosphoroamidocyanidate ethyl ester), is present in small quantities only at TEAD (*7,10*). Physical and chemical characteristics of these agents are presented in Table 1 and their chemical structures are shown in Figure 1.

Organophosphate (OP) nerve agents were designed specifically to cause incapacitation or death in military use and are particularly effective because of their extremely high acute toxicity. This acute toxicity is three to four orders of magnitude greater than most of the chemically similar OP pesticides, whose acute toxicological endpoints are much the same (*15*). The probability of an inadvertent release with off-site consequences during current storage or any disposal alternative considered in the CSDP-FPEIS is extremely low, being estimated to range from 1 in 10^{-4} to 1 in 10^{-10} (*7,9*). A credible risk, for purposes of CSDP planning, is conservatively considered to be one with a probability of one in 100 million or greater ($\geq 10^{-8}$) (*16*). Some of the release scenarios considered in the CSDP-FPEIS include exposure of Army personnel and a few extend to off-site populations. Effects on individuals could range from none to life threatening, depending on factors such as the type and concentration of agent released, the duration of exposure, individual variations in sensitivity, and the availability of antidotes, decontamination, and treatment capability. Some low-probability scenarios could result in catastrophic aggregate effects (i.e., > 1000 fatalities). One alternative considered, and rejected in the FPEIS, was continued storage of the agents for 25 years. This option is estimated to entail a higher number of potential fatalities from credible accidents than on-site disposal (*17*).

An analysis of the toxicity of each nerve agent in the stockpile was performed as part of evaluating the on- or off-site destruction options (*7*). Watson et al. (*18*) and Carnes and Watson (*9*) summarized the results of that analysis. This review is the third in a series of articles in *EHP* addressing health effects issues related to stockpile destruction. In the first, Munro et al. (*10*) evaluated nerve and vesicant agent antidotes, decontamination procedures, and treatment protocols for use in a civilian context. In the second, Watson and Griffin (*19*) detailed the toxicity of vesicant agents, with particular attention to mustard agent carcinogenicity. The present review documents essential information on nerve agent toxicity that is useful to civilian medical personnel and emergency planners involved in preparation for stockpile disposal at each community.

We first review briefly the general features of the nerve agents, signs and symptoms of exposure and mechanism of action,

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We acknowledge the contributions of Frederick Sidell, who provided expert consultation and reviewed the sections on acute nerve agent toxicity. Mohamed Abou-Donia, Thomas Bucci, Nada Grujic Chang, Jack Dacre, Marvin Goldman, Sanford Leffingwell, Dennis Opresko, and Barry Wilson also provided valuable information and/or review and comments. We acknowledge with much appreciation the special contributions of Jeffrey Ryman (Figure 1) and Emily Copenhaver (Figures 2 and 3) in producing electronic versions of these figures. This research was sponsored in part by the Office of the Program Executive Officer, Program Manager for Chemical Demilitarization, Department of the Army, under Interagency Agreement 40-1354-83, and by the Office of the Assistant Secretary of the Army under Interagency Agreement 1769-1354-A1 with Oak Ridge National Laboratory under Martin Marietta Energy Systems, Inc. contract DE-AC05-84OR21400 with the U.S. Department of Energy.

Received 3 November 1992; accepted 15 November 1993.

biochemical indicators of exposure, and metabolism. We then present indices of acute toxicity of the nerve agents alone and in combination, followed by information on potential delayed and persistent effects of acute exposure. These endpoints include delayed neuropathy, psychological and EEG changes, and cardiac effects. We next review results of studies on chronic or subchronic systemic toxicity, carcinogenicity, genotoxicity, teratogenicity, and reproductive toxicity. Finally, we discuss the implications of the varied acute toxic effects of nerve agent exposure for protection of the general public, as well as emergency and medical personnel.

General Features and Mechanism of Nerve Agent Action

According to Harris and Paxton (*20*), GA, or tabun, was the first nerve agent developed for chemical warfare; it was discovered in late 1936 and produced in large scale by 1942 (*20,21*). Subsequent G agents such as GB are both more toxic than GA and also more resistant to hydrolysis. GA contains cyanide instead of fluoride (see Fig. 1). It is more volatile than VX (see Table 1). Agent GA is stored in relatively small quantities in bulk at only one rather remote continental U. S. site (TEAD) (Table 1). Thus, concerns about public health hazards presented by GA in the course of the CSDP are relatively minor compared with those of GB and VX.

Because of its volatility, GA is primarily an inhalation hazard; it tends to disperse rapidly and is not likely to be a contact or ingestion hazard. However, GA is less volatile than GB (Table 1) and would be expected to remain on the skin and in the environment somewhat longer. Although it is not as persistent as VX, under certain weather conditions (light breeze, 20°C or 68°F) GA can remain in the environment from 1 to 4 days (*22*). Agent GA differs from other G nerve agents in some of its biochemical effects on the brains of exposed animals and also in the rarity of GA-induced convulsions, even at lethal doses (*23*).

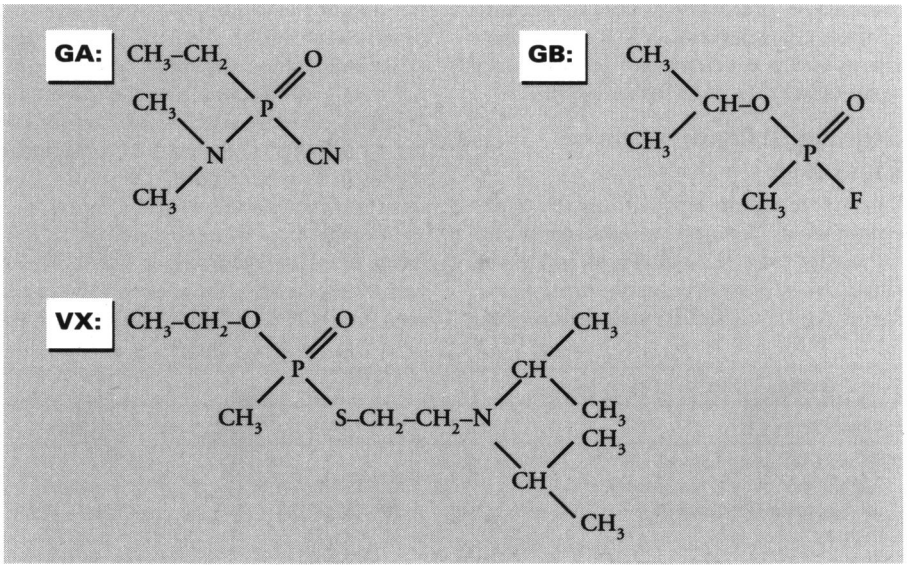

Figure 1. Molecular structures of the OP nerve agents GA, GB, and VX [adapted from Dacre (*13*) and Gordon et al. (*14*)].

Agent GB, or sarin, a fluorine-containing OP (Fig. 1), is the most studied of the three nerve agents considered in this analysis. Because of its high volatility and expected rapid dispersion, GB is the agent of greatest concern for acute inhalation exposures in an unplanned release at those sites housing it (ANAD, BGAD, PBA, TEAD, and UMDA; Table 1). Because of its high volatility, GB is not a great concern from the standpoint of reentry to a previously contaminated area. GB is somewhat less effective as a skin penetrant than as an inhalant because it evaporates so rapidly from the skin.

Agent VX, a sulfur-containing OP (Fig. 1), is, by any route of exposure, the most potent of all the nerve agents discussed here (Table 2). When compared to the G agents, VX is more stable, more resistant to detoxification, less volatile, more efficient at skin penetration, and

Table 1. Chemical and physical properties, location, and type of chemical munitions (*11,12*)

	GA, tabun	GB, sarin	VX
Chemical name	Ethyl-*N*, *N*-dimethyl phosphoramidocyanidate	Isopropyl methyl phosphonofluoridate	*O*-ethyl-*S*-(2-diisopropyl aminoethyl) methyl phosphonothiolate
Chemical formula	$C_5H_{11}N_2O_2P$	$C_4H_{10}FO_2P$	$C_{11}H_{26}NO_2PS$
Chemical abstract no.	77-81-6	107-44-8	50782-69-9
Molecular weight	162.1	140.1	267.4
Description	Colorless, odorless liquid	Colorless, odorless liquid	Colorless, odorless liquid
Melting point, °C	-50	-56	-39 (calculated)
Boiling point, °C	245	158	298
Density (liquid, g/mL, 25°C)	1.08	1.09	1.0083
Volatility (mg/m^3, 25°C)	610	2.2×10^4	10.5
Solubility, water (g/l)	98 (25°C) (miscible)	Miscible	30 (25°C) 75 (15°C) (miscible <9.4°C)
Solubility, other	Very soluble in most organic solvents	Readily soluble in organic solvents	Readily soluble in organic solvents
Storage location[a]	TEAD	ANAD, BGAD, PBA, TEAD, UMDA	ANAD, BGAD, NAAP, PBA, TEAD, UMDA
Munition type[b]	TC	P, R, B, C, TC	P, R, M, ST, TC

[a]TEAD, Tooele Army Depot; ANAD, Anniston Army Depot; BGAD, Blue Grass Army Depot; PBA, Pine Bluff Arsenal; UMDA, Umatilla Depot Activity; NAAP, Newport Army Ammunition Plant.
[b]B, bombs; C, cartridges; M, mines, P, projectiles; R, rockets, S, shells; ST, spray tanks; TC, ton containers.

more environmentally persistent. Because of these characteristics, VX is more effective as a skin penetrant and lethal contact agent rather than as an inhalation threat.

Signs and Symptoms of Exposure

The nerve agents are among the most potent of all chemical warfare agents and are highly toxic in both liquid and vapor form. In vapor or aerosol form, nerve agents can be inhaled or absorbed through the skin or the conjunctiva of the eye; as a liquid, they can be absorbed through the skin, conjunctiva, and upper gastrointestinal tract (*38*). Because they are essentially colorless, odorless, tasteless, and nonirritating to the skin, their entry into the body may not be perceived by the individual until grave signs and symptoms appear.

Within seconds after exposure to low levels of nerve agent vapor, local effects may be observed in the eyes and the respiratory system of humans. Depending on the agent and the dosage, the local ocular effects may be a constriction of the pupils of the eye (miosis) lasting only several days or a prolonged miosis persisting for many weeks (*39,40*), pain, and/or dim vision (*29*). Respiratory effects may include bronchoconstriction, excess secretion in airways, wheezing, and labored breathing (*29*). The time of onset of moderate systemic effects depends in part on the route of exposure; it is within seconds to a minute or two after inhalation, within 45

Table 2. Acute toxic levels of nerve agents

Exposure route	GA	GB	VX	VX (aerosol)
Inhalation, LCt_{50} (mg-min/m^3)				
Human, mild activity (estimated)	135–150 (*11,21*)	70 (*11*)	30(*11*)	20–50 (*24*)
Human, resting (estimated)	200–400 (*11,22*)	100 (*11,22,25*)	36 (*22*)	
Monkey	187 (*11*)	74 (*11*)	~50 (*11*)	
Dog	320 (*11*)	60 (*11*)	15[a] (*11*)	
Rabbit	960 (*11*)	120 (*11*)		25[a,b] (*11*)
Guinea pig		180 (*11*)		8, 30[a,c] (*11*)
Rat	450 (*11*)	220 (*11*)	17[a] (*11*)	
Mouse		240, 310[d] (*11*)	40 (*11*)	7 (*11*)
Dermal, airborne LCt_{50} (mg-min/m^3)				
Human, clothed (estimated)		15,000 (*11*)	60, 3,600[e] (*11*)	75, 300[f] (*24*)
Human, bare skin (estimated)	~20,000–40,000 (*11,22*)	12,000(*11*)	6, 360[e] (*11*)	
Dog, clipped			4.6, 89[g] (*11*)	3.5, 31.8[h] (*11*)
Rabbit, clipped		2,000 (*11*)	8.3, 28[i] (*11*)	124, 180 (*11*)
Rabbit, clipped and clothed			539[j] (*26*)	
Dermal, liquid LD_{50} (mg/kg)				
Human (estimated)	14–21 (*27*)	24 (*11*)	0.04 (*28*), 0.14 (*29*)	
Monkey, shaved	9.3 (*11*)		~0.065 (*11*)	
Pig, clipped		115.9 (*11*)	<0.40 (*11*)	
Dog, depilated	~45 (*11*)	10.8 (*11*)	0.054 (*11*)	
Cat, depilated		6.2 (*11*)	0.012 (*11*)	
Rabbit, depilated	3 (*11*)	4.4 (*11*)	0.025, 0.205[k] (*30*)	
Rat, depilated	12.6 (*11*)	2.5 (*11*)	0.10 (*11*)	
Mouse, depilated		1–9.2 (*11,31*)	0.046 (*11*)	
Intravenous LD_{50} (mg/kg)				
Human (estimated)	0.014 (*21*)	0.014 (*11*)	0.008 (*11*)	
Monkey	~0.05 (*11*)	0.020 (*11*)	0.0084 (*11*)	
Goat		0.015 (*11*)	<0.005 (*11*)	
Dog	0.084 (*11*)	0.010 (*11*)	0.0063 (*11*)	
Cat		0.015-0.018 (*11,32*)	~0.0025 (*11*)	
Rabbit	0.063 (*11*)	0.0147 (*33*)	0.0084 (*11,28*)	
Rat	0.07 (*11*)	0.045 (*11*)	0.0079 (*11*)	
Mouse	0.311(*34*)	0.07–0.113 (*34–36*)	0.0141 (*11*)	
Oral LD_{50} (mg/kg)				
Rat	1.06 (*11*)	0.10 (*11*)		
Incapacitating dose, ICt_{50} (mg-min/m^3)				
Human, inhalation, mild activity (estimated)	100 (*22*)	35–72 (*11,22,27*)	24 (*11*)	5–15 (*24*)
Human, inhalation, resting (estimated)	300[j] (*26*)	75 (*27*)		
Human, percutaneous, clothed (estimated)		8,000 (*11*)		30–150[f] (*24*)
Effective dose-miosis, ECt_{50} (mg-min/m^3)				
Human, inhalation (estimated)	0.9 (*11*)	2–4 (*25*)	0.09 (*37*)	
Effective dose-tremors, ECt_{50} (mg-min/m^3)				
Human, inhalation (estimated)[l] (*25*)		28 (*25*)	1.6 (*36*)	
Effective dose-GI signs, ED_{50} (mg/kg)				
Human, bare skin, 18-24°C (estimated)			0.0314 (*11*)	
No-effects dose-miosis (mg-min/m^3)				
Human, inhalation (estimated)		0.5 (*25*)	0.02 (*37*)	
No-effects dose-tremors (mg-min/m^3)		4.0 (*25*)		

[a]Only head exposed.
[b]Wind speed 0.01 mph.
[c]Wind speed 15 and 0 mph, respectively.
[d]Active, resting, respectively.
[e]Masked, wind speed 15 and 1 mph, respectively.
[f]Masked, wind speed 10 and 0 mph and particle size 15, 5 µm, respectively.
[g]Wind speed 20 and 0 mph, respectively.
[h]Wind speed 15 and 5 mph, respectively.
[i]Wind speed 8 and 0 mph, respectively.
[j]Clothed in cotton sateen over cotton t-shirt.
[k]Bare, clipped; and clothed, unclipped, respectively.
[l]Calculated from LCt_{50}/ECt_{50} = 2.5 at 15 l/min-breathing rate

min after ingestion, and within 2–18 hr after application on the skin (*38,41*). Exposure to lethal doses, however, can lead to collapse within seconds and death within 10 min after a single deep inhalation (*21*). After short-term (acute) exposure, mild systemic effects may last for several hours to days, whereas moderately severe symptoms can last for 1–6 days. During the recovery period, symptoms may recur intermittently, particularly after physical exertion (*38*).

The toxic actions of nerve agents are due primarily to their ability to inhibit acetylcholinesterase (AChE), an enzyme responsible for the breakdown of the neurotransmitter acetylcholine (ACh). The result is excessive ACh accumulation at synapses, where only minute quantities of ACh are needed for transmission. Acetylcholine overstimulation of the portions of the nervous system that control smooth muscle, cardiac muscle, and exocrine glandular function results in the following signs: drooling, increased bronchial secretions, bronchoconstriction, miosis, excessive sweating, vomiting, diarrhea, abdominal cramping, involuntary urination, and cardiac arrhythmias. In addition, ACh overstimulation of the central nervous system (CNS) may result in headache, anxiety, restlessness, irritability, giddiness, insomnia, nightmares, EEG changes, or even convulsions and coma, depending on the agent and the dosage (*38*). Finally, ACh accumulation affects the nerves controlling skeletal muscle, resulting in a dose-dependent generalized weakness that increases with exertion, as well as muscle twitching and fasciculation, cramping, and even flaccid paralysis.

Respiratory failure, the immediate cause of death in nerve agent exposure, is an example of an effect that occurs as the result of ACh accumulation at several sites in the nervous system. Depression of the brain's respiratory center, neuromuscular block of the respiratory muscles, bronchial constriction, and increased lung secretions are all factors contributing to nerve agent-induced respiratory failure; the relative importance of each depends on the species studied, the nerve agent, and the dosage used (*42–48*).

Recent interest has developed in the acute behavioral toxicity of nerve agents. In this relatively new field of investigation, animals are tested for changes in motor and learning behavior after exposure to the compound of interest. Karczmar (*49*) has listed the CNS effects, including behavioral and mental health effects, that have been observed with several anticholinesterase chemicals. To date, the number of these effects that can be ascribed to nerve agent exposure is limited. In most cases, motor effects in animals appeared at levels of exposure that caused mild (some salivation, fine tremors) to moderate (excessive salivation and weeping, generalized tremors) toxic effects (*36,50–54*). However, some animal tests indicate acute effects on learning behavior at exposure levels below those that cause signs of nerve agent poisoning (*51,53*). Results of such studies must be interpreted with much caution (*55*). Applicability to humans is suggested by preliminary work in volunteers exposed to VX, demonstrating that performance on a number facility test was impaired to a statistically significant extent ($p < 0.01$) in conjunction with minimal or absent physical signs and symptoms (*56*).

Mild to severe human exposures to nerve agents have been associated with other mental and emotional effects. These range from giddiness and loss of ability to concentrate through anxiety, tension, and irritability, to withdrawal, depression, insomnia, and nightmares (*56,57*). Such effects and associated EEG changes were experienced in concert with the onset of nausea and other symptoms in the case of GB, or earlier in the case of VX. Some mental and emotional effects may persist for hours, days, or weeks, depending partly on the severity of exposure.

Agent-induced ACh accumulation generates side effects that involve action on other CNS neurotransmitter systems (e.g., norepinephrine, dopamine, γ-aminobutyric acid). Numerous biological effects result (*58–61*) including hypothermia in rats (*61,62*), prolonged analgesia in mice (*63*), and brain and cardiac lesions in animals surviving high doses of nerve agents (*64,65*). The interplay of the various neurotransmitters within the nervous system probably results in these varied side effects (*45,66*), although nerve agents may exert direct effects on these same noncholinergic systems (67–69).

Biochemical Indicators of Nerve Agent Exposure

Despite new knowledge derived in animals as to the novel cholinergic and noncholinergic effects of nerve agents and related organophosphates, it is still widely accepted that inhibition of AChE is the primary cause of acute toxic responses to nerve agent exposure in humans. For this reason, attempts have been made to measure blood cholinesterase (ChE) activity as an indicator of the magnitude of nerve agent exposure and/or the severity of clinical signs and symptoms or to monitor the return of blood ChE function as an index of recovery. Only in the case of systemic effects is there a reasonably good correlation with the degree of ChE inhibition.

Two types of ChE activity can be measured in blood, red blood cell AChE (RBC-ChE), and nonspecific plasma ChE (butyryl ChE or pseudocholinesterese). Systemic effects are seen in about 50% of exposed volunteers when RBC-ChE is 20–25% of normal baseline (a depression of 75–80%) (*56,57,70-72*). Monitoring of RBC-ChE activity is theoretically preferred because this cholinesterase is similar to the AChE found at the nerve synapses. RBC-ChE, however, is replenished only with the formation of new RBCs in the case of GB (*57*), while it spontaneously reactivates (1% per hour) in the case of VX (*54*). Furthermore, recovery of function or cessation of signs and symptoms occurs well before RBC-ChE levels show much recovery, especially after GB exposure (*57*). Thus, recovery of RBC-ChE activity does not reflect the time course of recovery of AChE activity in the tissues. As a result, monitoring of RBC-ChE activity is of questionable utility in assessing recovery from nerve agent (particularly GB) exposure in individuals for whom baseline RBC-ChE values are unavailable (see below) (*71*).

Plasma ChE measurement is less relevant than RBC-ChE activity; the inhibition of plasma ChE, which has no known biological function, may not reflect actual AChE inhibition (*73*). For example, agent VX causes significantly less inhibition of plasma ChE than AChE (*57,72,74*). Agent GB also preferentially inhibits RBC-ChE (*75*), although not to the same extent as VX. Furthermore, plasma ChE is more labile than RBC-ChE, being affected by gender, age, and oral contraceptive use (*76*), as well as genetic determinants, disease states, nutritional status, hormonal changes, race, and circadian patterns (*77,78*). Plasma and RBC-ChE do serve a protective function, complexing with nerve agent and thus reducing the concentration of free nerve agent available to complex with tissue AChE (Fig. 2).

Because of the variability of blood ChE activity (both plasma and RBC) in unexposed individuals, it is difficult to determine conclusively from a single test whether a person has had a recent exposure to a cholinesterase inhibitor, especially if the exposure is minor (*79,80*). Yager et al. (*81*) found the RBC-ChE intraindividual coefficient of variation to be 10.0% and that of plasma ChE to be 14.4%. With one prior measurement of baseline ChE activity in an individual, a 15% RBC-ChE depression is the least that can be reliably detected compared to a 20% decrease from baseline for plasma ChE (*80*) (Table 3). When individual baseline blood AChE activity is known and is compared with the post-exposure activity level, a dose-dependent relationship can be demonstrated between AChE inhibition and dosage at a limited

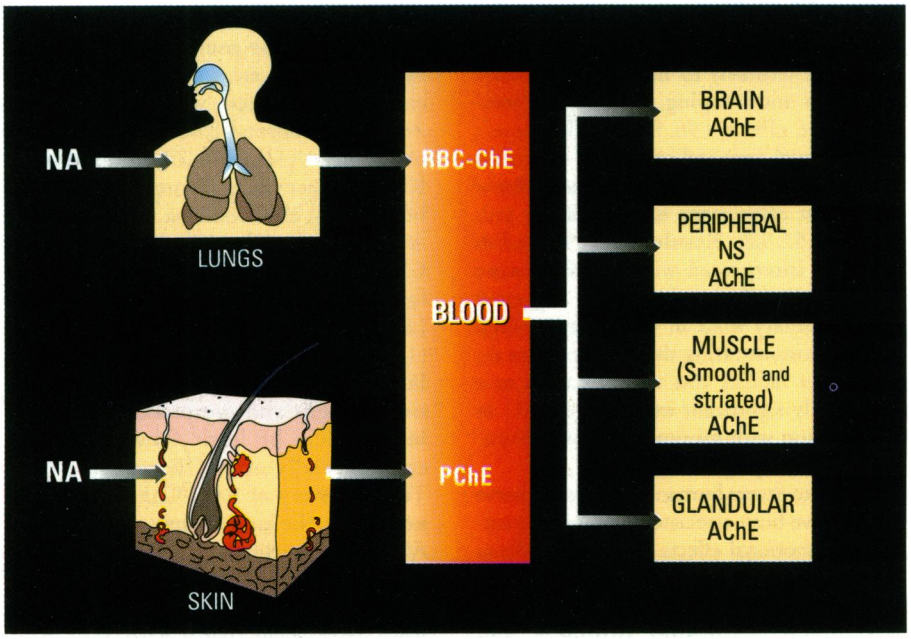

Figure 2. Plasma and RBC-cholinesterase (ChE) provide a buffer to neurotoxic action by complexing with nerve agent, thus reducing the concentration of free nerve agent available to move into tissue compartments and inactivate synaptic or myoneural acetylcholinesterase (AChE). PChE, plasma cholinesterase; NA, nerve agent; NS, nervous system.

range of acute sublethal doses (*56,57*). Doses of GB and VX required to depress human RBC-ChE activities by 50% (ChE_{50}) are presented in Table 4. Brain AChE inhibition and the degree of toxicity show a better correlation in that GA and GB injected into rats produced a dose-dependent inhibition of brain AChE, with lethal doses producing > 90% inhibition (*61*). Although brain AChE activity may reflect the dose response to nerve agent exposure more closely than blood AChE, human brain AChE monitoring can be done only in an invasive manner and thus has no practical application in assessing human exposure.

Metabolism

The detoxification or breakdown of GA within the body proceeds at a low rate (*27*), by way of the enzyme diisopropylfluorophosphatase (formerly termed tabunase), which has been identified in several species including man (*83*). Agent GB is detoxified in certain animal species by the plasma enzyme carboxylesterase, formerly called aliesterase. Carboxylesterase combines rapidly with GB and prevents it from interacting with AChE. In rats, 10 min after intravenous (IV) injection of radiolabeled GB, approximately 70% of the plasma activity was bound to large protein molecules identical to carboxylesterase (*84*). Pretreatment of rats with tri-orthocresylphosphate (TOCP), a weakly anti-ChE organophosphorus compound that irreversibly blocks carboxylesterase, resulted in a six- to eightfold enhancement of GB toxicity (*85*). Additionally, more GB was found in the brain, muscles, kidneys, and lungs and less GB in the plasma of TOCP-pretreated rats as compared to rats that received no pretreatment. Similar carboxylesterase modification of GB toxicity has been observed in guinea pigs and mice, although guinea pig plasma carboxylesterase binding capacity for GB is lower than that of rat plasma (*86,87*). The presence of carboxylesterase in rodent plasma may by itself account for the relative resistance of mice and rats to GB toxicity compared with other animal species (see Table 2 for LD_{50} values). Human plasma does not contain carboxylesterase. Grob and Harvey (*57*) calculated that there is very little detoxification when GB is injected into the human bloodstream. This major difference in the detoxification of GB between rodents and humans highlights the uncertainty of estimating human LD_{50} values from data obtained for rodents.

Table 3. Minimal differences required for recognition of statistically significant depression of individual human plasma and red cell cholinesterase activity values[a]

No. of preexposure estimations	% Difference from baseline	
	Plasma	Cells
1	19.9	15.3
2	17.3	13.3
3	16.3	12.5
4	15.7	12.1
5	15.5	11.9
10	14.7	11.3
∞	14.1	10.9

[a]Adapted from Callaway et al. (*80*), with permission.

Metabolism studies of GB have been carried out in dogs and mice. Metabolism studies in dogs demonstrated that the nearly exclusive product of GB detoxification is isopropyl methylphosphonic acid (*88*). This compound accounted for the majority of GB activity found not only in plasma and urine, but also in brain tissue, suggesting that brain, like a variety of tissues from several mammalian species, can hydrolyze GB (*88*). A rapid hydrolysis of intravenously injected GB occurred in mice, such that less than 10% of the GB found in the tissues was nonhydrolyzed within 1 min (*36*). This rapid hydrolysis of GB may be again due to plasma AE, but this hypothesis has not been established. A question remains as to the relevance of extrapolating to humans from any metabolism studies using a species (mouse) that is resistant to the toxic effects of GB.

Acute Toxicity

Because human exposure data are not available on the lethal doses of the nerve agents discussed here, animal toxicity data have historically been extrapolated to develop human dose estimates. The dose or exposure levels of GA, GB, and VX that result in 50% lethality (LD_{50}, LCt_{50}) in several species by various routes of exposure are presented in Table 2. The route of exposure is important because there are differences in absorption and/or degradation with different avenues of entry into the body. Although the IV route is not relevant to accidental exposure of man, inclusion of these data in Table 2 illustrates the intrinsic toxicity of the agents without individual or species variations in absorption and is useful for comparison. Table 2 also includes estimates of doses, based on animal data, that could cause 50% mortality in human populations exposed by some of these same routes. Comparisons between species (particularly comparisons of animal data with human estimates) are possible since the LD_{50} values (or the estimated values) are given on a milligram of agent administered per kilogram of body weight (mg/kg) basis. Table 2 also contains the estimated human median incapacitating concentration-time product (ICt_{50}), effective dose or concentration-time product (ED_{50}, ECt_{50}) [also termed minimum effective dose in the U. S. Army Chemical Agent Data Sheets (*11*)], and no-effects dose for each nerve agent. Unfortunately, the source document (*9*) does not define what is meant by incapacitation.

Agent GA

As mentioned previously, the human doses for lethality (LD_{50}) or incapacitation (ICt_{50}) provided in Table 2 are merely estimates (*11,22*). This is evident in a compar-

ison of the inhalation incapacitating Ct product (ICt_{50}) of 300 mg-min/m^3 and the range of 200–400 mg-min/m^3 for the lethal dose (LCt_{50}) for GA in resting humans (breathing 10 l/min); the incapacitating dose falls within the range for the lethal dose. The degree of incapacitation associated with this dose is not defined in the source (*11*), but likely is severe, meaning unconscious and convulsing.

Agent GB

GB is a very rapidly acting toxicant; there is little difference between the 15-min and the 24-hr lethal dose for animals by IV injection (Table 2) (*28*). GB has been thought by some to act primarily on the peripheral nervous system; however, respiratory arrest induced in cats by an IV dose equivalent to one-half the feline LD_{50} (*48*) was mediated through effects on the central nervous system. GB is very efficient at producing central respiratory arrest in guinea pigs and cats at IV doses too low to cause an effect on the respiratory muscles (*32*). Thus, the primary effects of GB appear to be on the CNS.

Like all other nerve agents, GB combines with and inhibits AChE, resulting in the accumulation of ACh. From studies in which small quantities of GB were injected directly into the bloodstream of human volunteers, Grob and Harvey (*57*) calculated that about 75% of GB combined with AChE in the muscle, about 22% with blood ChE, and about 3% with AChE in brain and liver. The inhibition of blood ChE results in no toxic effect; rather, it is the GB inhibition of brain and muscle AChE that causes the symptoms of nerve agent exposure. Within the muscles of cats, GB caused a dose-dependent inhibition of AChE activity; however, no simple relationship existed between AChE inhibition and alteration of muscle function (*89*).

Once GB is in the blood, it can penetrate the blood–brain barrier. Cholinesterase inhibitors vary in their ability to pass through this barrier, a property that has been related to the lipid solubility of the compound (*90*). Within the brains of mice injected intramuscularly with GB, Bajgar (*91,92*) observed regional differences in AChE inhibition. He concluded that the differences in AChE inhibition were due to regional differences in GB penetration rather than to a differential selectivity of GB for AChE in specific parts of the brain. Studies of isolated, blood-perfused *in situ* dog brains administered GB via intracarotid arterial injection (*93*) and of the brains of dogs after IV injection of GB (*88*) also showed regional differences in AChE activity. Studies in rats demonstrated that more than 94% of apparent GB bound to AChE in the brains 30 min after injection is actually the GB metabolite isopropyl methylphosphonic acid (*94*).

Table 4. Human RBC-ChE_{50} values for GB and VX administered by various routes

	GB	Reference	VX	Reference
Inhalation	20 mg-min/m^3 0.004 mg/kg	(*25*) (*82*)	5 mg-min/m^3	(*37*)
Percutaneous, vapor	—		100 mg-min/m^3	(*37*)
Percutaneous, undiluted liquid	—		0.034 mg/kg (12 hr) 0.029 mg/kg (24 hr)	(*72*) (*72*)
Intravenous	0.003 mg/kg	(*57*)	0.001 mg/kg, 0.0011 mg/kg	(*74*) (*56*)
Oral	0.01 mg/kg	(*57*)	0.0023 mg/kg[g]	(*56*)

Mechanisms other than (or in addition to) AChE inhibition appear to be responsible for the observed toxicity of GB to the brain. In rats, Harris et al. (*95*) reported that 51% of the GB found in the brain was bound to sites other than AChE. In studies of spontaneous recovery from central respiratory failure in guinea pigs, respiratory recovery did not correlate with recovery of brainstem AChE levels (*44*). Adams and his colleagues concluded that the recovery occurred through a desensitization of the ACh receptors to the excess ACh, but it is also possible that AChE inhibition was not actually responsible for the initial respiratory failure. GB causes a number of noncholinergic effects in the brain, including effects on other neurotransmitters and enzymes. Most effects are too detailed to discuss individually in this analysis, but all emphasize the point that GB does much more than simply inhibit AChE in the brain (*23,58,59,61,67,96,97*).

Data on human responses to GB come from accidental exposures and from limited studies on low doses of GB given to volunteers. In one incident of accidental exposure to GB vapors (estimated at 0.09 mg/m^3 for an undefined duration), two men had significantly lowered RBC-ChE for 80–90 days (one showed depression to 19% of baseline activity, the other to 84% of baseline) and extreme miosis that persisted for 30–45 days, but no other signs or symptoms of nerve agent poisoning (*39*). Other accidental inhalation exposures to GB with similar recovery times for RBC-ChE activity and miosis were described by Sidell (*40*). In one, the individual manifested severe symptoms and required respiratory assistance and extended hospitalization after cleaning a GB-contaminated area while wearing defective protective gear. In the other case, three workers who were in an area with a leaky GB storage container suffered temporary symptoms, such as transient mild respiratory distress, together with marked miosis and RBC-ChE activity depression. The RBC-ChE depression required 3 months for full recovery; the miosis (measured in the dark) recovered in 30–60 days.

Grob and Harvey (*57*) reported the effects in humans of administered low doses of GB. When either 0.003 or 0.005 mg/kg of GB was injected directly into an artery in the arm of one volunteer, Grob and Harvey observed some initial local effects (reduction in grip strength, tremors after exercise) followed by systemic effects, including many of the symptoms listed earlier. These doses, which correspond to 21% and 36% of the estimated human IV LD_{50}, resulted in RBC-ChE activity reductions to 52% and 28% of original activity (i.e., depressions of 48% and 72%) and plasma ChE activity reductions to 61% and 42% (i.e., depressions of 39% and 58%), respectively.

After combining with a ChE molecule, the agent-ChE complex may either spontaneously dissociate (resulting in reactivation of the ChE) or "age," in which case the agent-ChE complex becomes resistant to reactivation by an oxime antidote. Aging is thought to result from stabilization of the nerve agent-ChE complex by loss of an alkyl or alkoxy group (Fig. 3). In agreement with Grob and Harvey's work (*57*), Sidell and Groff (*56*) observed little, if any, spontaneous reactivation of RBC-ChE after GB administration to volunteers. Furthermore, the GB-ChE complex aged at a moderate pace, with aging 50–60% complete 5 hr after GB infusion (*56*).

When GB was given orally to 10 volunteers, approximately 3.5 times as much GB (in mg/kg) was needed to produce the same degree of plasma or RBC-ChE activity depression as previously observed with intra-arterial injection (see Table 4) (*57*). With the oral administration, Grob and Harvey noted a narrow margin between doses that produce mild signs and symptoms and those that produce moderately severe effects. They also noted that, after the disappearance of signs and symptoms, an increased susceptibility (in terms of type and severity of responses) remains to further GB exposure within 24 hr of the first exposure. Anorexia, nausea, and chest tightness were among the first symptoms

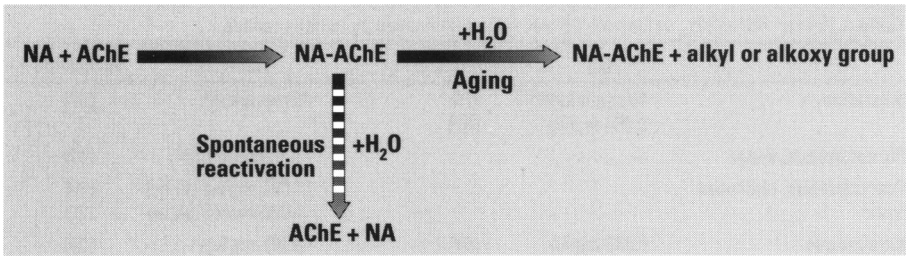

Figure 3. The nerve agent–acetylcholinesterase (AChE) complex may undergo either spontaneous reactivation by hydrolysis or stabilization ("aging") by loss of an alkyl or alkoxy group; stabilization proceeds at a faster rate than hydrolysis and therefore predominates. In humans, the GB–AChE complex is 50–60% aged by 5 hr, whereas VX ages more slowly, with only 40% aged at 48 hr after exposure (*56*).

reported; abdominal cramping, vomiting, and diarrhea were among later effects; miosis was not observed after oral administration. The possibility of oral exposure of the population to GB is remote because GB dissipates rapidly under most environmental conditions. Only when temperatures are 0° or less can GB persist for a few hours as a ground contaminant (*22,98*).

As mentioned previously, GB vapor is less effective as a toxic skin penetrant than as an inhalant. The estimated human LCt_{50} (clothed, resting) for dermal toxicity is 150 times higher than the estimated human LCt_{50} for inhalation (Table 2). Fielding (*28*) summarizes information from several sources, some still classified. Rapid evaporation from the skin is the primary factor in the relatively low dermal toxicity of GB; if evaporation is prevented (i.e., by covering the exposed skin with a cup), the toxicity of GB increases almost 100-fold (*99*). Another factor limiting the dermal toxicity is the reaction of GB with skin constituents, which attenuates the amount of GB that reaches target tissues (*100*). Fats such as lanolin and lard have been shown to enhance the skin penetration of GB, probably by dissolving the agent and by preventing evaporation (*28*). Mechanical abrasion of rabbit skin increased GB dermal toxicity 100-fold (*101*). Fielding (*28*) relates a tragic incident that illustrates the wide individual variability in dermal sensitivity to GB. Seventeen of 18 men exposed dermally to 200 mg of GB (12% of the estimated dermal LD_{50} for a 70 kg man) through two layers of clothing showed no signs or symptoms of GB poisoning; the eighteenth man died shortly after the onset of exposure, despite immediate treatment when signs of nerve agent poisoning appeared.

In a review of GB toxicity, McNamara and Leitnaker (*25*) state: "Absorption through the conjunctiva causes local effects but negligible systemic effects." Grob and Harvey (*57*) instilled 0.0003 mg GB in the eyes (conjunctival sacs) of volunteers and noted a marked miosis that began at 10 min and slowly diminished over a period of 60 hr. At a dose of 0.0009 mg, the pupillary constriction that occurred was near maximal for 72 hr and did not disappear until after 90 hr. In this study, miosis was measured in the light; other studies in which it was measured in the dark showed it persisted for weeks. No depression of blood ChE activity was noted at either dose level. In studies on GB applied to the conjunctival sac of guinea pigs, a rapid dose-dependent depression of AChE activity in the iris and cornea was noted with a lesser inhibition of AChE in the retina (retina required 10 times the iris dose to achieve the same AChE inhibition), but no examination was made of RBC-ChE depression or other systemic effects in the treated guinea pigs (*102*). However, ocular LD_{50} values are available for several animal species that are equivalent to the LD_{50} values for subcutaneous injection (*11*). This suggests that systemic effects are possible with GB absorption through the conjunctiva and possibly the cornea of the eye.

Studies of the retention and absorption of GB vapors by resting or exercising men demonstrated that the inactive men retained a higher percentage of the inhaled GB (*82*). Under similar exposure conditions of time and concentration, however, the active men received a larger dose of GB because of their greater air intake.

In determining the lowest concentration of GB that produces a biological effect, miosis provides a sensitive indicator for nerve agent exposure in humans. Questions, however, cloud the validity of the estimated no effects (0.5 mg-min/m^3) concentration-time product (Ct) for miosis by GB (Table 2). The basis for this determination by McNamara and Leitnaker (*25*) is found in a report by Johns (*103*) of pupil diameter response in volunteers exposed to low atmospheric concentrations of GB (maximum Ct = 6 mg-min/m^3 where t_{max} = 20 min). We consider the data insufficient to confidently predict concentrations of GB that would cause miosis in none of the population (no-effects level). The Johns study (*103*) was not designed to determine a no-effects level; it is not clear how McNamara and Leitnaker (*25*) derived their no-effects value of 0.5 mg-min/m^3 from Johns's data. We consider the true no-effects level likely to lie below 0.5 mg-min/m^3. The lack of raw data and absence of measures of variability in Johns's (*103*) report hinder precise reanalysis. Estimates of a human no-effects level for VX, as discussed below, were based in part on these for GB; the VX estimate consequently suffers from a similar question of reliability.

Agent VX

A contributing factor to the high toxicity of VX may be its preferential reaction with AChE. Unlike the G agents, VX depresses RBC-ChE activity significantly more than plasma ChE in humans (*56*); the result is that more VX is available to react specifically with the target enzyme, AChE. Less VX is required than GB to reduce RBC-ChE 50% below baseline levels in humans by all routes of administration for which data are available (see Table 4).

Once inside the body, VX not only inhibits AChE activity but also reacts directly with the ACh receptors and other neurotransmitter receptors (*68,97,104*). Rickett et al. (*105*) have briefly reviewed some of the evidence for effects at the receptor level. Although GA and GB may react with the ACh receptor in a manner similar to ACh itself (*106*), results of preliminary studies suggest that VX may counteract the effects of ACh, acting as an open channel blocker at the neuromuscular junction (*105*). The clinical significance of these effects is doubtful, however, because the concentrations of anticholinesterase needed to exert effects in ionic channels *in vitro* are many times the LD_{50} *in vivo*.

Two other features of VX toxicity are worthy of mention. First, in contrast to observations on GB, the VX-RBC-ChE complex has been found to undergo a significant degree of spontaneous reactivation in humans. In a study by Sidell and Groff (*56*), spontaneous reactivation of human RBC-ChE proceeded at a rate of about 1%/hr over the first 70 hr after IV administration of VX.

A second feature of VX toxicity is the lack of aging or stabilization of the agent-ChE complex and the relative ease of reactivation of VX-poisoned enzyme by oxime antidotes in humans (*56*). By 48 hr after exposure, no aging was observed. The VX-ChE complex was more easily reactivated by oxime antidote at all times up to 48 hr after exposure (when the experiment was terminated) than was GB-ChE.

Estimates are available for human lethal inhalation doses of VX in both aerosol (small particles) and vapor (gas) phases (Table 2). Animal inhalation data are available primarily for VX aerosol. In most cases, only the animals' heads and

not their total bodies were exposed, so as to limit the skin absorption of VX. The mouse LCt_{50} values for both vapor and aerosol were obtained with total-body exposure; in the case of VX aerosol, skin absorption appears to contribute to the total toxicity. The estimated human LCt_{50} values are equivalent for vapor and aerosol. However, it would probably be difficult to achieve a high vapor concentration of VX because of its low volatility; therefore, it is likely that a longer exposure to VX vapor would be necessary to achieve the same endpoint.

It should be borne in mind that the VX LD_{50} values for humans have been derived from mathematical models, extrapolations from animal data, and estimates from sublethal experimentation in humans. Many of the original reports in which these human values were derived are confidential and unavailable for open-literature review. In general, AChE activity levels have been used as indicators of VX toxicity in humans. However, extrapolation to LD_{50} estimates from AChE activity determinations may have little meaning because of the poor correlation between AChE activity and toxicity in animals and wide variations in RBC-ChE levels at which toxic effects occurred in human studies (*56,74*).

Dermal absorption is a more likely route of VX exposure than inhalation; moreover, dermal toxicity is more likely to occur from the absorption of VX aerosol or liquid than from the vapor. The LCt_{50} estimates for dermal absorption are established by exposure of animals to VX vapor or aerosol in a special chamber in which only the body is exposed. The animals were often shaved, clipped, or depilated before exposure to approximate human skin exposure, and the wind speed within the chamber was varied to simulate a range of meteorological conditions.

Although wind speeds of 20 mph may never be encountered in an unplanned release of VX, it is important to realize that wind speed can significantly increase the dermal toxicity of VX. A 20-mph wind speed resulted in a 20-fold reduction in the dog LCt_{50} values when compared with tests conducted in still air (89 versus 4.6 mg-min/m^3; Table 2), and the rabbit LCt_{50}, when determined with an 8-mph wind speed (8.3 mg-min/m^3), was 3.4 times lower than that obtained in still air (28 mg-min/m^3) (Table 2). Another way of determining VX dermal toxicity in animals is to apply liquid VX directly to the bare skin. The LD_{50} values for skin absorption are similar for monkey, pig, dog, cat, rabbit, and mouse (Table 2).

Although the animal data summarized in Table 2 primarily show the influence of wind speed, other factors affecting the dermal LCt_{50} values include particle size and degree of skin exposure (clothed or bare). Krackow (*24*) calculated dermal LCt_{50} values for men wearing gas masks so that only the neck, ears, hands, and wrists were exposed to aerosol particles ranging in size from 5 to 15 μm at wind velocities from 0 to 10 mph. Under these conditions, a lower LCt_{50} (75 mg-min/m^3) was estimated for the larger particles with the higher wind velocity, while an LCt_{50} of 300 mg-min/m^3 was for the smaller particles at the lowest wind speed. Other data of Krackow suggest that, with 10-μm particles and 20-mph winds (or 20-μm particles and 10-mph winds), the LCt_{50} might be as low as 10 mg-min/m^3.

The human dermal LCt_{50} values listed in Table 2 for VX vapor are based on VX vapor containing 2-μm particles (*11*). These exposure conditions represent a hybrid vapor/aerosol situation. Fielding (*28*) considers that the dermal LCt_{50} values for VX vapor and VX aerosol would be similar under conditions of high concentrations in air and short exposure times. With lower vapor concentrations, Fielding (*28*) notes that "doubt has been expressed regarding the dermal lethality of VX vapor to humans in view of the possibility that the (systemic metabolism and) excretion rate may be greater than the skin-absorption rate."

The amount of skin surface exposed to VX vapor or aerosol is of obvious importance. Clothing is estimated to reduce by 10-fold the dermal vapor toxicity of VX (see Table 2). Not all body areas, however, are equally permeable to VX. The doses of VX necessary to cause 70% inhibition of AChE when applied to equal areas of the human cheek, forehead, abdomen, and volar surface (i.e., palm side) of the forearm have been estimated to be 0.0051, 0.0112, 0.0318, and 0.04 mg/kg, respectively (*107,108*). The differences in absorption are important in evaluating studies in which the forearm is exposed and extrapolations are made to total-body exposure. Craig et al. (*108*) measured the dermal absorption of liquid VX through the cheeks and the forearms men at environmental temperatures ranging from -18° to 46°C. The fraction of the applied dose that penetrated in 3 hr ranged from 3.5% at -18°C to 31.9% at 46°C for the cheek and from 0.4% at +18°C to 2.9% at 46°C for the forearm. Wide individual differences in RBC-ChE depression were seen for both skin sites and most doses tested; in some cases, responses ranged from 0 to 100% depression (*108*). The wide range of individual responses to dermal VX exposure, caused in part by differences in penetrability of the skin in various parts of the body, makes the prediction of a human dermal VX LD_{50} value difficult. Thus, ranges are given rather than single values.

Because skin can act as a storage depot for VX with movement from this depot promoted by increased temperature, the authors suggest that cooling of the skin surface after dermal exposure to VX can delay absorption until treatment is possible. Also note that immediate decontamination should be a particularly effective procedure for dermal VX exposure because of the slowness of its skin penetration. However, if decontamination is delayed until 3 hr after exposure, significant lowering of RBC-ChE continues after decontamination (*108*). Specific decontamination procedures following nerve agent exposure can be found in Munro et al. (*10*).

The estimated IV LD_{50} for humans (0.008 mg/kg) is similar to that determined for many animal species, with the major exception of the mouse, which is less sensitive. Low doses of VX (0.001 mg/kg, IV) were administered to volunteers to assess correlation of dose with the degree of AChE inhibition or the presence of clinical signs and symptoms. By injecting VX directly into the bloodstream, the wide differences in individual skin absorption observed in other studies (*108*) can be bypassed. In four men who received 0.001 mg/kg VX in a 4-hr infusion, good agreement was observed between the individual percent decrease (50%) in RBC-ChE activity compared with preinfusion AChE levels (*74*). (It is not possible to compare the range of the absolute AChE values because these were not given.) In another study, reported by Sidell and Groff (*56*), a group of 34 men were given doses ranging from 0.0012 to 0.0017 mg/kg in an attempt to find an IV dose of VX that would cause 75% inhibition of RBC-ChE levels. In this dose range, most subjects had transient symptoms of lightheadedness and some experienced nausea and vomiting, with the most prominent effects occurring 1 hr after injection, when the RBC-ChE inhibition was maximal. No miosis was observed, even with 90% inhibition of RBC-ChE activity. A dose of 0.0015 mg/kg produced 75% inhibition of the baseline AChE levels; linear regression analysis of the dose–response curve gave 0.0011 mg/kg as the estimated dose causing 50% decrease in RBC-ChE activity (see Table 4).

There is a paucity of data on the oral toxicity of VX, despite the fact that the demonstrated environmental persistence of this agent makes ingestion a relevant route for human exposure. VX can persist on leaf surfaces in an undegraded form, so that animals grazing on contaminated vegetation can ingest VX. In a study on VX persistence in soil after shell bursts, 46 days after contamination sufficient VX re-

mained to kill 4 of 10 guinea pigs fed grass from the contaminated area [Dewey and Fish (*28,109*). In sheep accidentally exposed in winter to VX-contaminated vegetation, clinical signs of toxicity persisted for at least 3 weeks (*110*). Slight ChE depression was noted in newly introduced sheep grazing the suspect area 2 months after the VX release (*111*). The only animal oral LD_{50} value available for VX is 0.1 mg/kg for rats (Table 2).

In 32 human volunteers given single oral doses of VX in water, a dose of 0.004 mg/kg caused a 70% reduction in RBC-ChE levels (*56*). This oral dose is about three times the human IV dose needed to cause a similar level of RBC-ChE inhibition. The oral ChE_{50} value calculated by Sidell and Groff (*56*) from the dose–response curve obtained in their study is 0.0023 mg/kg (see Table 4). At oral doses ranging from 0.002 to 0.0045 mg/kg, only a few subjects (5/32) suffered any gastrointestinal signs or symptoms, and there were no changes in heart rate, blood pressure, or pupil size in any of the subjects. Eating 30 min before drinking the VX solution appeared to enhance the RBC-ChE inhibition; tap water (as compared with a 5% dextrose solution) seemed to retard the anti-RBC-ChE activity. In an earlier study, volunteers were given four oral doses/day of VX in drinking water for 7 days (concentration about 0.05 mg/l in four 500-ml portions; individual daily dose of 0.00143 mg/kg) (*112*). No signs or symptoms of toxicity were observed although RBC-ChE was 40% of baseline by day 7.

Estimates have also been made for human ICt_{50} either by inhalation or by skin absorption of VX aerosol or vapor (Table 2). The ranges in these estimates are due in part to different test conditions (i.e., varied particle sizes in the case of aerosols and different wind velocities). Fielding (*28*) notes that these ranges may also depend on what is meant by incapacitation.

Estimates have been made for the lowest air concentration of VX that produces miosis, one of the more sensitive indices of human exposure to the vapors of anticholinesterase compounds. The estimated ECt_{50} found in Table 2 for pupillary constriction by VX is an extrapolation from the derived value for GB in humans (*25*). To obtain the VX ECt_{50} for humans, the estimated ECt_{50} for miosis in humans exposed to GB was first compared with the minimum dose of GB that causes miosis in rabbits, and the assumption was made that man is twice as sensitive as the rabbit. This factor of two was then applied to the concentration of VX that produces pupillary constriction in rabbits to arrive at the minimum concentration of VX that would be expected to cause miosis in humans (*37*). Similarly, the VX no-effects doses for miosis and for tremors are based on extrapolations from derived values for GB. Because these VX estimates are used to determine presumed safe levels for human exposure to VX, more research is needed to determine whether these minimum and no-effects values are credible.

The effects of acute VX exposure on mood and mental function are similar to those of GB. Kimura et al. (*74*) reported the results of the first experimental human exposures to VX. One subject became irritable, reported headache, spoke less clearly, and became confused and then irrational and agitated after receiving 0.00212 mg/kg VX IV over 5.5 hr when whole-blood ChE activity reached about 10% of baseline. Six others received 0.001 mg/kg VX IV over periods of 1.75–4 hr; only one subject reported headache and discomfort. Bowers et al. (*41*) reported transient depressive effects on mental functioning and mood (as correlated with ChE depression and gastrointestinal symptoms) in 93 adult male volunteers percutaneously to VX. [Bowers et al. (*41*) identify the agent only as EA-1701; Krackow (*24*) identifies this code with VX.] The psychological effects were usually seen well before the onset of gastrointestinal symptoms in those subjects who experienced both types of effects.

Sidell and Groff (*56*) reported a study in which 66 volunteers received VX either IV or orally. Doses ranged from 0.0012 to 0.0017 mg/kg IV and 0.0020 to 0.0035 mg/kg orally. The 0.0015 mg/kg IV group suffered a significant decrement in mental performance on a number facility test (the higher dose groups had been pretreated with scopolamine and were not monitored for changes other than in RBC-ChE activity). The effect was seen only in the first hour after VX injection. Those receiving VX by the oral route showed little or no indications of CNS effects and fewer gastrointestinal effects despite generally lower RBC-ChE activities. Thus, the authors suggested that the nausea and vomiting in the IV group were probably centrally mediated.

Comparative Toxicity of GA, GB, and VX

The relative potency of GA, GB, and VX varies with the route of exposure. Inhalation or percutaneous absorption of vapor or aerosol demonstrates that VX is more toxic than GB, which is more toxic than GA (i.e., VX > GB > GA). The dermal toxicity ranking is VX > GA > GB, while the ranking based on estimates of IV toxicity is VX > GA = GB. These differences relate to varying physical, chemical, and toxicological properties among the nerve agents. Agent VX, for example, is not only much less volatile than the G agents, it is not detoxified in the skin and combines little, if at all, with plasma cholinesterases. Thus, VX is more readily available to inactivate tissue AChE.

The human inhalation toxicity of GA vapor is approximately half that of GB (Table 2); this difference is well supported by the animal data. GA appears to be more toxic to the ciliary muscles of the eyes than GB because constriction of pupils occurs at a lower concentration of GA [i.e., minimum effective doses of 0.9 and 2–4 mg-min/m^3, respectively (*11*)]. The estimated LD_{50} for GA toxicity in humans by skin absorption is roughly equivalent to the estimate for GB, and the human IV LD_{50} estimate for GA is equal to that for GB. The equivalencies of these estimates are not necessarily supported by the animal data, but no discussions of the bases for the human estimates are given in the source documents (*11,21*).

Although GB is less toxic than VX by a variety of exposure routes, GB may actually be more toxic than VX at the neuromuscular junction. When GB or any one of several V agents related to VX was applied directly to the isolated rat diaphragm at the neuromuscular junction (thereby eliminating factors such as absorption efficiencies and attenuation differences), GB was found to be twice as potent as the V agents (*113*). Intravenous infusion of GA, GB, or VX in cats at the rate of one LD_{50} per 15 min demonstrated that 0.5 LD_{50} of GB was sufficient to induce respiratory arrest, whereas 1.25 and 15 LD_{50} doses were needed for GA and VX, respectively (*48*). These differences reflect the rapidity of the toxic action of GB compared with VX and the somewhat higher toxicity compared with GA.

In comparison with GB human exposure estimates, VX is estimated to be approximately twice as toxic by inhalation, 10 times as toxic by oral administration, and approximately 170 times as toxic after skin exposure (*114*). Under conditions favorable for skin penetration, VX can be about 1000 times as toxic as GB in rabbits (*28*). The evaporation of VX from the skin is almost negligible, whereas GB evaporates in a matter of minutes (see Table 1 for comparative volatility data). Agent GB penetrates the skin more rapidly than VX, but VX undergoes virtually no degradation as it slowly penetrates the skin; thus, more of this compound is able to reach the bloodstream (*115*). Whereas GB skin penetration in rabbits appeared to be complete by 30 min (*100*) [with a penetration efficiency of nonevaporating GB calculated to be only 0.04% (*28*)], complete penetration by VX, with essentially 100% of the skin dose reaching the circulatory system, required about 4.5 hr (*116*). *In vitro* stud-

ies suggest that VX can penetrate in unaltered form through the epidermis and dermis of the skin, penetrate through the nerve membranes, and can accumulate within the nerve cells (*117*).

A number of investigators have reported the distinctly slower toxic action of VX as compared with the G agents, as well as a slower rate of recovery (*105,118*). This delay cannot be attributed only to slower skin penetration because a slower response is also observed when VX is administered intravenously (*48,56*). With GB there is essentially no difference between the 15-min and 24-hr lethal IV dose; with VX there is an approximate twofold difference (*28*). It is important, therefore, in determining LD_{50} and LCt_{50} levels for VX to allow enough time to accurately assess the toxicity. Although the biological basis for this delay is not fully understood, Fielding (*28*) hypothesized that the larger molecular size (see Fig. 1) and different solubility characteristics of VX may cause it to diffuse more slowly than G agents through tissues and cell membranes to the target tissues.

Mixtures

Both GB and VX are stored at several of the stockpile sites (LBAD, ANAD, PBA, TEAD, and UMDA). While munitions containing a given agent are placed in segregated bunkers, igloos, or storage buildings and, likewise, ton containers are segregated by agent type, the stockpile sites contain limited areas of contiguous rows of bunkers or other storage units containing unlike agents. While the probability of an accident such as an airplane crash into one of these areas of adjacent storage units resulting in release of more than one agent type is extremely low, such a release is considered here for the sake of completeness.

Thus, the question arises as to toxic effects of a GB-VX mixture if these agents were simultaneously released. In the only study found to date that addresses this issue, GB and VX were administered simultaneously and sequentially to mice (*119*). When the agents were administered as a mixture of 0.5 LD_{50} of each agent (GB = 95 µg/kg, VX = 9 µg/kg), the resulting mixture had an LD_{50} lower than one LD_{50} of either agent alone, meaning that the total effect was more than additive. When a 0.5 LD_{50} dose of VX was administered 1 hr before a 0.5 LD_{50} dose of GB, brain and blood AChE were depressed less than by sequential administration of two 0.5 LD_{50} doses of GB given 1 hr apart. Thus, VX had a protective effect on blood and brain AChE depression produced by GB. However, when the nerve agents were administered in reverse order (0.5 LD_{50} of GB before 0.5 LD_{50} of VX), blood AChE inhibition was greater, but brain AChE inhibition was less than that induced by two serial 0.5 LD_{50} doses of VX.

Approximately 50-fold potentiation of toxicity with the administration of certain combinations of OP insecticides [EPN, *O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate and malathion] has been described (*120*); however, with other insecticide combinations [malathion and ronnel; compound 4072 (Dermaton) and dichlorvos; ronnel and dichlorvos] there was no potentiation of AChE inhibition in the dog (*121*). Further investigation is needed to quantify the possible interactions of toxic mixtures of nerve agents or combinations of nerve agents and pesticides, especially those relevant to the CSDP.

Delayed and Persistent Effects of Acute Nerve Agent Exposure

Public concern has been expressed regarding the induction of organophosphorus-induced delayed neuropathy (OPIDN) by the OP nerve agents in the U. S. stockpile. Other possible delayed or persistent effects of concern include cardiac dysfunction, psychological effects, and EEG abnormalities.

The OPIDN syndrome is characterized by a delay of 5–30 days, followed by some initially mild symptoms, such as weakness, tingling, and muscle twitching in the legs. A flaccid paralysis eventually develops, first in the toes and then progressing to the hands and thighs. Depending on dose, the paralysis is usually persistent; recovery is generally slow and incomplete. Some 16,000 cases of OPIDN were reported in 1930–31 among individuals in the southern United States who drank an illicit alcoholic extract ("Jamaica Ginger" or "Ginger Jake") that was contaminated with TOCP, a weak anticholinesterase OP ester (*122,123*). Thousands of others have suffered from TOCP-induced OPIDN as a consequence of ingesting contaminated food oils (*124,125*). A limited number of people have also developed delayed neuropathy in response to other OP compounds, mainly the OP insecticides malathion, parathion (*122*), methamidophos (*126*), mipafox, a fluorine-containing OP (*127*), isophenfos (*128*), and probably leptophos (*129*). Other OPs implicated causally in human OPIDN induction are listed in a recent review by Lotti (*130*) and include dichlorvos, EPN, trichlorfon, and trichlornat. Delayed neuropathy induction is associated with 70–80% inhibition of a specific protein, neuropathy target esterase (NTE; formerly termed neurotoxic esterase). The function of NTE and role, if any, in the mechanism of OPIDN induction is unknown. Recent reviews by Johnson (*131*), Abou-Donia and Lapadula (*132*), and Lotti (*130*) summarize much of what is known about NTE. Abou-Donia and Lapadula (*132*) propose a mechanism involving phosphorylation of Ca^{2+}/calmodulin kinase II, increasing its activity and causing disruptive effects on cytoskeletal proteins in neuronal tissue. Thus, although NTE inhibition may be a marker of OPIDN-inducing potential, it may play no role in OPIDN induction.

Human beings are one of the most sensitive species for OPIDN induction; hens are equivalently sensitive and have been used widely to test chemicals for OPIDN-inducing potential (*133*). To date, human beings are known to be significantly more sensitive than hens to OPIDN induction by only one chemical, methamidophos (*126,130*). Lotti (*130*) attributed this greater human sensitivity to a higher rate of spontaneous reactivation of methamidophos-inhibited human AChE, and the availability of assisted ventilation to human beings (but not hens).

Ratios of anticholinesterase activity and NTE-inhibiting activity *in vitro* for hen tissue (AChE I_{50}/NTE I_{50}) and human tissue are similar (*133,134*). *In vivo* toxicity ratios (LD_{50}/neurotoxic dose) for the hen correspond well with the *in vitro* AChE I_{50}/NTE I_{50} ratios. Thus, it is likely that *in vivo* assays in hens and *in vitro* human and hen enzyme activity ratios are at least qualitatively predictive for human OPIDN-inducing potential (*133*).

Although the data are often sparse on the other delayed health effects of nerve agents per se, an expanding field of literature on delayed health effects of OPs, particularly insecticides, exists (*49,66,35*). Information from this literature is included, particularly where there are data gaps for nerve agents.

Agent GA

OPIDN. Agent GA has not been shown to produce OPIDN, but it appears that it may have the potential to do so under conditions highly unlikely for human exposure. Agent GA at extremely high doses inhibits NTE both *in vitro* (*136*) and *in vivo* in antidote-protected chickens (*134*). Johnson et al. (*137*) showed that GA produces the aged or unreactivatable form of inhibited NTE that is often associated with induction of OPIDN. Results of *in vitro* assays of GA potency against bovine AChE and hen NTE suggested that doses of 100–150 times the LD_{50} would be necessary to induce OPIDN *in vivo* in hens (*14*). Tests of GA in antidote-protected chickens at 120 times the subcutaneous (SC) LD_{50} dose of 0.610 µmol/kg (*14*) [two 6 mg/kg doses/day intramuscular (IM); total of 12 mg/kg/day] elicited mild neuropathic signs in one of two surviving

hens (*138*). Despite this observation, neuropathy was not observed in survivors of a single dose (12 or 15 mg/kg, IM) in that same study (*138*) nor in survivors of single doses (12 mg/kg, IM) or two 12-mg/kg doses (total dose, 24 mg/kg, IM) (one surviving hen) (*137*). Willems et al. (*138*) concluded that even higher doses of GA would be needed to produce the fully developed clinical signs of delayed peripheral neuropathy in chickens. Henderson et al. (*139*) found no effect of GA on NTE activity in chicken with or without atropine protection given single IM injections of 0.125 mg/kg; results of 90-day studies using 0.07 mg/kg IM 5 days/week in atropine-protected hens were negative for behavioral or histopathologic signs of OPIDN. Furthermore, dosing male and female CD rats without atropine protection at 1, 1/2, and 1/4 times the MTD (0.1125, 0.05625, and 0.02813 mg/kg IP, respectively) 5 days/week for 13 weekss (90-day study) revealed no effect on brain NTE activity (*140*) and no clinical evidence of neuropathy (*141*). The higher two doses used were sufficient to depress plama and RBC-ChE activities significantly and result in transient clinical signs of OP toxicity in some animals (*141*).

It appears that GA doses many times the LD_{50} may be necessary to induce OPIDN in humans. If unprotected human populations were ever exposed to doses this great, there would be few survivors. There are no data to support OPIDN induction in humans at less-than-lethal doses of GA. Thus, OPIDN induction is not a relevant concern for GA exposure.

Agent GB

OPIDN. Agent GB at high doses has been shown to cause OPIDN in chickens, a particularly sensitive species for this endpoint (*14,142–144*). This effect required doses 30–60 times the chicken IM LD_{50} (0.025–0.05 mg/kg) (*142,143*) in birds protected from death by prior injection of antidotes. A review by Abou-Donia (*129*) documents the induction of OPIDN by GB only in chickens and only at supralethal doses (1 mg/kg). More recently, Crowell et al. (*145*) failed to observe significant decreases in brain, spinal cord, or lymphocyte NTE in atropine-protected hens 24 hr after single administration of GB as sarin I by gavage at 1, 3.3, and 6.6 times the maximum tolerated dose (MTD) (0.61, 0.2, and 0.4 mg/kg) or sarin II at 1, 1/2, and 1/4 the MTD (0.28, 0.14, and 0.07 mg/kg). The positive control, TOCP, was effective at lowering all types of NTE. Repeated doses of GB (by gavage at 1/3, 1/6, and 1/12 MTD for 42 days) in atropine-protected hens failed to result in signs of OPIDN (*146*), despite exposure sufficient to cause signs of acute toxicity.

Little evidence for GB-induced neuropathy has accrued from studies in mammals. For example, agent GB failed to induce OPIDN in cats either at a supralethal dose, 1 mg/kg, SC, in atropine-physostigmine-protected animals (compared to the LD_{50} dose of 0.035 mg/kg, SC), or at multiple low-dose exposures adding up to the LD_{50} (*147,148*). The low doses (0.0035 mg/kg/day for 10 days or 0.007 mg/kg/day for 5 days, SC) generated no signs of cholinesterase poisoning. Agent GB (sarin I) also failed to induce OPIDN in CD rats exposed by gavage five times per week for 13 weeks (90 days) at doses ranging from 0 to 0.3 mg/kg/day (the MTD) (*149*). A 15% decrease ($p < 0.05$) in NTE was seen only in the high-dose female group. Sarin II in similar experiments also failed to induce neuropathy in rats at doses up to the MTD, and no effects on NTE were seen at any dose (*150*). It should be noted that the rat, unlike the cat, is relatively insensitive to full OPIDN induction (*130,132*) and variably sensitive to histopathological damage. A study of the effects of high (convulsive, 5 μg/kg) or multiple small-dose GB exposures of rhesus monkeys on EEG patterns showed no difference in behavior between exposed and control animals examined both at 24 hr and 1 year after dosing (*151*). No signs of atoxia were noted. Some primates are considered resistant to OPIDN induction, but others are susceptible (*129*).

A recent report (*152*) of a small study in eight female Swiss albino mice suggests early changes characteristic of OPIDN induction resulting from low-dose GB exposure. The animals were exposed 20 min/day for 10 days, whole-body, to 5 mg/m^3 GB (17% of LC_{50}, 600 mg/m^3/min, for this strain). This exposure regimen resulted in 27% inhibition of blood AChE and 19% inhibition of brain AChE but caused no signs of anti-AChE toxicity. By day 14 after onset of exposure, the mice displayed mild signs (slight ataxia, muscle weakness of limbs, twitching), NTE inhibition of brain, spinal cord, and platelets, and light or moderate axonal degeneration in the spinal cord. Mipafox caused more pronounced changes in positive control animals. At present we know of no reports indicating that other groups have tried to replicate these results in this or any other mouse strain. Further work should be done to verify these results in view of the lesser sensitivity of the mouse to OPIDN induction by TOCP (*153*).

Bidstrup et al. (*127*) reported that another fluorine-containing OP, mipafox, induced delayed neuropathy in two chemists (a male and a female) after occupational exposure sufficient to cause severe acute toxic effects. A third worker who developed less severe acute symptoms failed to exhibit OPIDN. No dose information was presented, but substantial exposure over a period of several days was documented. Davies et al. (*142*) tested 36 alkyl OPs and found that the 17 compounds that caused delayed neuropathy contained fluorine. However, the possession of a fluorine atom by an OP compound does not, by itself, indicate neurotoxic potency, as a number of fluorine OP compounds have been tested and found to lack such activity (*144*).

Although many human volunteers (246 individuals) (*144*) have been exposed to GB by a variety of routes, no reported instances of OPIDN are known, either from the experimental studies (*144,154*) or from accidental exposures of more than 200 individuals (Leffingwell SS, personal communication). The doses ranged from those causing no signs and symptoms and no detectable decrease in RBC-ChE activity to doses causing moderate or severe signs and symptoms and RBC-ChE activity depression of as much as 90% below normal baseline levels (*57,155*). Most of the accidental GB exposures were very mild and, while formal long-term follow-up was not done, no employee reported signs of OPIDN after returning to work, nor did they report such symptoms on subsequent contacts with the medical staff. Six severe GB exposures are known, and the U. S. Army Medical Research Institute of Chemical Defense is not aware of any evidence for OPIDN having developed in any of those cases (Sidell FR, personal communication).

In summary, while the possibility of a human developing OPIDN in response to a supralethal dose of GB cannot be ruled out, the major concern would be immediate treatment to prevent death. There is no evidence of GB causing OPIDN in humans, nor is there current evidence for this effect resulting from low-dose GB exposure (lower than those resulting in acute toxic effects) in any species other than the mouse.

Psychological effects. Acute exposure to GB has been shown to cause both transient and prolonged changes in psychological function. Evidence is available from several cases of accidental exposure in which the doses are unknown but effects can be categorized as severe or moderate (*56,156, 157*). At least some of the persistent changes may have resulted from brain damage caused by GB-induced convulsions (*156*). Agent GB induction of transient depressive emotions, insomnia, excessive dreaming, and nightmares have been observed in volunteers in the absence of seizure activity (*155*). Grob and Harvey (*57*) reported similar effects, as well as EEG changes that persisted for 4–18 days after oral administration of GB to 10 vol-

unteers for 1–4 days. Repeated doses were sufficient to produce 85% depression of plasma ChE activity and more than 97% depression of RBC-ChE activity. Associated physical signs and symptoms were described as moderately severe but fell short of convulsions. Occupationally exposed workers exhibited similar signs and symptoms after low-level exposures to G agents; in some cases, effects persisted beyond 3 days (*158,159*).

Sidell (*71*) considers that mild psychological changes resulting from nerve agent exposure to be more common than ordinarily recognized and to occur even in a small fraction of individuals experiencing few or no other signs of exposure; effects may persist from days to weeks. Sidell (*71*) also points out that the psychological effects can delay fitness for return to any work requiring full cognitive function and rapid decision-making.

That GB doses sufficient to cause acute toxic effects may also result in long-term psychological changes is further suggested by a recent study of workers previously acutely intoxicated by OP insecticides. This study documents persistent insecticide effects similar to those of nerve agent exposure on mental function and emotional state. Savage and colleagues (*135*) evaluated 100 individuals who had experienced 1 or more documented episodes of acute poisoning on average 9 years earlier (in at least 80% of the cases by parathion, methyl parathion, or malathion, dose unknown). These individuals showed mild but statistically significant deficits in intellectual ability, academic skills, abstract thinking ability, and speed and coordination on motor skill tests in comparison to matched controls. They evidenced more depression, irritability, confusion, and tendency to withdrawal than controls on an inventory by relatives and perceived themselves to have areas of difficulty with memory, thinking ability, and use of language.

Organophosphate insecticides are sequestered in body fat and gradually mobilized from these depots to a greater extent than the OP nerve agents as evidenced by their longer time course of recovery and need for repeated treatment with atropine (*160,161*). Thus, OP insecticides may be more likely than nerve agents to cause CNS effects and to induce changes persisting longer than those possibly induced by OP nerve agents.

EEG effects. Duffy and colleagues reported subtle long-term changes in human brain function after acute GB exposure (*162,163*). In these studies, exceedingly subtle changes in EEG patterns and increases in rapid eye movement (REM) sleep were observed at 1–6 years after accidental exposure to GB sufficient to cause acute signs and symptoms and to lower RBC-ChE by at least 25% below baseline. Statistically significant EEG changes were detected only by computer analysis in a group comparison of exposed workers with control subjects; trained neurologists were unable to distinguish by visual inspection between EEGs of exposed and unexposed individuals. Thus, the EEG changes are not considered clinically significant. Some of the workers studied by Duffy and colleagues (*162,163*) had been studied earlier by Metcalf and Holmes (*164*), who also reported on EEG, psychological, and neurological changes in persons exposed to OPs including insecticides and nerve agents. When the EEG patterns of the exposed OP worker group were compared with the EEGs of a control group of workers who had no exposure or access to OPs, minimal group differences were observed, consisting mainly of increased medium-voltage irregular θ waves, usually during drowsiness [for details of EEG spectra differences, see Duffy et al. (*162*)]. Note that, as in the study by Duffy and collegues (*162,163*), these EEG changes were evident a year or more after exposure—during this time the workers had no other known OP exposure and showed no blood ChE activity depression. Comparing the "highly exposed" worker group to the "minimally exposed" worker group, Metcalf and Holmes (*164*) found memory, concentration, and sleep disturbances, as well as subtle EEG changes (not clinically significant) and minor motor coordination deficits. The Metcalf and Holmes report does not clarify whether the GB exposures of the subjects were recent, nor whether the persistent EEG changes could be correlated with the observed persistent psychological changes.

After the observation of EEG changes in GB-exposed workers, a study was carried out in monkeys in an attempt to substantiate these long-term EEG effects (*151*). The monkeys were given either a single dose of GB (0.005 mg/kg, IV) that produced overt toxic signs or 10 smaller doses (0.001 mg/kg, IM, at weekly intervals) that resulted in no clinical signs. In both the acute and subchronic exposure groups, increases in β activity were observed in the spontaneous cortical EEG patterns up to 1 year after exposure.

No difference in gross behavior was observed between treated and control animals. Another important finding from this study was that, at 1 year, there were greater differences in the EEG patterns of the animals that received the series of smaller doses (with no resulting clinical symptoms) than in the animals receiving the single dose. Because the total dose in the series was twice that of the single dose, this suggests it is the total amount of GB received and not the induction of clinical effects that determined the degree of EEG alteration.

In summary, clinically insignificant EEG changes and increases in REM sleep were observed in the worker group exposed 1–6 years earlier to levels of GB sufficient to cause signs of toxicity. Changes were more evident in the worker group with more recent exposures or more than one episode of exposure (*163*). Although some workers in the same population had experienced psychological changes, this study did not address any possible correlation between EEG changes and psychological effects. Thus, the meaning of subtle persistent EEG changes after GB exposure is unclear; there may be no meaningful behavioral or physiological correlates. Levels of GB exposure too low to cause acute toxic signs and symptons have not been tested for the ability to induce persistent EEG changes.

Cardiac effects. Another potential delayed effect of GB exposure is cardiac damage. In a study of OP insecticide poisonings, certain clinical effects such as cardiac problems often showed a delay in their onset (*160*). Agent GB has been shown to cause cardiomyopathy in rats in doses sufficient to cause convulsions in many of the animals (0.111–0.17 mg/kg, SC) (*65*). Cardiac lesions were seen only in animals that had convulsed with resulting brain lesions. The cardiomyopathy may result from CNS damage and consequent sympathetic overstimulation.

Agent VX

OPIDN. Agent VX shows no potential for inducing OPIDN (Table 5). In tests of the ability of nerve agents to inhibit NTE *in vitro*, VX was at least 1000-fold less active than agent GB (*14,136*). Three VX-related thiolates were also ineffective at *in vitro* inhibition of NTE (*14*). Single IM or SC injections of VX at 0.15 mg/kg (5 times the LD_{50}, IM) in atropine-protected chickens produced neither inhibition of NTE nor histological or behavioral evidence of OPIDN (*165*). A structurally unrelated fluoridothionate compound was neuropathic in an acute test in the chicken at 5 mg/kg, IM (*142*). The ability of VX to induce OPIDN has also been tested in antidote-protected chickens when injected IM at 0.04 mg/kg for 90–100 days. The results of this subchronic exposure test were negative; no behavioral signs or histological degeneration of spinal cord or muscles were produced, in contrast to effects seen in the positive controls exposed to diisopropylfluorophosphonate (DFP) (*166*). In summary, there is no indication that VX has any potential at low or high doses for the induction of OPIDN in human beings or other species either with

Table 5. Results of testing for NTE inhibition or OPIDN induction by VX and related compounds

Compound	Species	Route	Concentration range or dose	Duration	Results
VX	Hen NTE	*In vitro*	10^{-7} to 5×10^{-5} M	20 min, 37°C	Negative for NTE inhibition (*136*)
VX	Hen NTE	*In vitro*	Not specified	30 min, 37°C	Negligible NTE inhibition (*14*)
Compound 6[a]	Hen NTE	*In vitro*	2.3×10^{-4} M	20 min, 37°C	Negligible NTE inhibition (*14*)
Compound 7[a]	Hen NTE	*In vitro*	7.4×10^{-4} M	20 min, 37°C	Negative for NTE inhibition (*14*)
Compound 8[a]	Hen NTE	*In vitro*	7.4×10^{-4} M	20 min, 37°C	Negligible NTE inhibition (*14*)
VX	Hen NTE	IM or SC	0.15 mg/kg ($5 \times$ IM LD_{50})	1 injection	Negative for OPIDN induction (*165*)
VX	Hen NTE	IM	0.04 mg/kg	90–100 days	Negative for OPIDN induction (*166*)

Abbreviations: NTE, neuropathy target esterase; OPIDN, organsphorus-induced delayed neuropathy; IM, intramuscular; SC, subcutaneous.
[a]Compounds 6, 7, and 8 were closely related thiolate analogues of VX; structures but no names given in original source.

acute or long-term exposure.

Psychological effects and EEG changes. Delayed or persistent psychological effects of VX have not been reported; however, no accidental exposures such as those with GB are known (*156*), and evidence of long-term, low-dose exposures such as the occupational exposures to GB has not been found for comparison. It is not known whether long-term psychological effects could be produced by acute or chronic exposure. The potential for VX to induce long-term EEG changes has not been tested.

Cardiac effects.. Acute exposure to agent VX has been shown to cause cardiac arrhythmias in rats (0.012 mg/kg, SC) (*167*) and beagle dogs (0.0015, 0.003, or 0.006 mg/kg, SC; 0.25, 0.5, and 1.0 LD_{50}, respectively) (*168*) at doses too low to cause convulsions. The arrhythmias in rats were associated with a high incidence of mortality. The ventricular arrhythmias seen in beagles included a form (Torsade de pointes, a rapid, multifocal ventricular arrhythmia) that is rare but characteristic of cardiac abnormalities seen in OP insecticide-poisoned humans. Histological examination to evaluate the induction of cardiomyopathy was not performed in these studies. Whether VX has the potential to cause fatal arrhythmias in humans or long-term cardiac damage at high doses is not known, although cardiac arrhythmias were not observed in experimental studies on volunteers reported above (*56,74*).

Chronic Toxicity, Genotoxicity, Carcinogenicity, Teratogenicity, and Reproductive Toxicity

Agent GA

Information on the toxicological effects of GA is limited in comparison to that for GB and VX, but a number of studies have been sponsored by the U.S. Army Medical Bioengineering Research and Development Laboratory; results are summarized in Table 6. These include studies of subchronic toxicity in rats, teratogenesis testing in rabbits, and several types of short-term genotoxicity.

Subchronic toxicity. Male and female CD rats were given GA without atropine protection at 0.1125, 0.05625, 0.02813, and 0 mg/kg/day, 5 days/week, for 13 weeks (90-day study). Plasma and RBC-ChE activities were significantly depressed in the two higher dose groups. No evidence of systemic toxicity was observed at any dose other than the effects on cholinesterase activity. Clinical chemistry results and hitopathology examinations revealed no other toxicity (*140,141*).

Genotoxicity. In tests of mutagenicity, GA was weakly mutagenic in the mouse lymphoma assay and in the Ames test using *S. typhimurium* (*170*). Agent GA induced sister chromatid exchanges (SCE) *in vitro* in mouse cells but not *in vivo* in mice (*170*). Agent GA failed to induce unscheduled DNA synthesis (UDS) in rat hepatocytes and, in fact, depressed UDS with no evidence of cytotoxicity (*170*). Wilson et al. (*170*) concluded that GA is a weakly active mutagen.

Teratogenicity. New Zealand white rabbits were used to test GA for teratogenic activity and fetotoxicity. GA was administered SC at 0.1125, 0.05625, 0.02813 mg/kg on days 6–19 of gestation. The results were negative for teratogenic activity, and no fetal toxicity of any kind was seen at doses below those causing maternal toxicity (Bucci TJ, personal communication).

Agent GB

Chronic and subchronic toxicity. Weimer et al. (*169*) exposed beagle dogs, Sprague-Dawley/Wistar rats, ICR Swiss mice, and tumor-sensitive Fischer 344 rats and strain A mice to low concentrations (0.001 and 0.0001 mg/m^3) of airborne GB for 6 hr/day, 5 days/week for 4–52 weeks. Animals were observed daily for toxic signs; blood chemistry was monitored monthly in the dogs and at the time of euthanasia of rodents; gross and microscopic examination of tissue samples from all major organ systems was performed; body weights were monitored throughout the exposure period and body and organ weights for heart, lung, liver, kidney, and testes or ovary were recorded at necropsy. Cardiovascular function was monitored in the dogs. No evidence of acute or chronic toxicity was found at these low intermittent exposure levels. Blood activity of RBC-ChE was not depressed in any species at either GB concentration.

Bucci and Parker (*149,150*) reported the results of subchronic toxicity testing in which male and female CD rats were exposed to GB together with the stabilizers at the concentrations used in unitary weapons systems [tributylamine (GB type I or sarin I) and diisopropylcarbodiimide (GB type II or sarin II)]. The rats were administered GB at 0.3, 0.15, and 0.075 mg/kg by gavage 5 days/week for 13 weeks. Body weights were monitored throughout the study; blood was drawn at 1, 3, and 7 weeks and at euthanasia for hematology and clinical chemistry measures. At necropsy, gross and histopathological examination of 144 tissues and all lesions was performed. Although plasma and RBC-ChE activities were significantly depressed at all dose levels, investigators saw no evidence of hematologic abnormalities; they noted no liver, kidney, or muscle damage, nor effects on body weight gain or organ weight. Neither form of GB was associated with any type of neoplastic or nonneoplastic lesion except for infrequent evidence of sarin I-induced cerebral necrosis. This effect was not related to dose.

Carcinogenicity and genotoxicity. In the studies by Weimer et al. (*169*), groups of each rodent strain were held for an additional 6 months for observation of carcinogenicity. No increase in tumors was detected in either the tumor-sensitive rodent strain or any other test animals in response to 6 hr/day, 5 days/week exposure for up to 52 weeks. Although the results suggest that GB is not carcinogenic, the low doses and less-than-lifetime exposure and observation period preclude definitive interpretation of the study.

Negative results in genotoxicity studies of GB as summarized in Table 6 support the likelihood that GB is not carcinogenic. Agent GB did not induce mutations in the Ames test (*171*) nor in mouse lymphoma cells (*171*); it failed to induce SCE (*174*)

Table 6. Results of genotoxicity, carcinogenicity, and other toxicological testing of the nerve agents GA, GB, and VX

	GA		GB		VX	
Effect/test	Result[a]	Reference/comments	Result	Reference/comments	Result	Reference/comments
Carcinogenicity						
Rat			–	(*169*); results negative but test not definitive; low dose (0.0001–0.001 mg/m^3) exposure up to 52 weeks		
Mouse			–			
Genotoxicity						
S. typhimuirium (Ames)[b]	W+	(*168*); 0.2–200 mg/plate	–	(*171*); 0.0002–0.2 mg/100 µl; type I and II tested[c]	–	(*172*); tested in 5 strains at $2.7 \times 10\text{-}9$–1.093 mg/plate (*173*); tested in 5 strains at 0.00001–0.01 mg/ml
Saccharomyces cerevisiae[b]					–	(*173*); 0.025–0.1 mg/ml
Mouse lymphoma mutation[b]	W+	(*170*); tested at 5 concentrations (0.01–0.025 mg/ml) with activation; tested at 4 concentrations (0.01–0.1 mg/ml) without S-9 activation	–	(*171*); 0.05, 0.1, 0.2 mg/ml	–	(*173*); tested at 6 concentrations, 0.001–0.1 mg/ml
Sister chromatid exchange	W+ *in vitro*	(*170*); 0–0.2 mg/ml with and without activation; tested *in vivo* at 0.7 mg/kg IP	–	Type I and II tested[c]; (*174*); 1.4×10^{-3} M		
Unscheduled DNA synthesis	–	(*170*); tested at 4 concentrations (0.025–0.2 mg/ml without activation	–	Type I and II tested[c]; (*173*); 3.0×10^{-4} to 2.4×10^{-3} M[d]		
D. melanogaster (sex-linked recessive lethal test)					–	(*172*); 5×10^{-6} and 0.004 mg/m^3
Teratogenicity						
Sheep					–	(*111*); accidental exposure
Rabbit	–	(Bucci T, personal communication)	–	(*176*)[f]	–	(*173*); 0.00025–0.004 mg/kg SC, gd 6-19
Rat			–	(*176*)[f]	–	(*173*); 0.00025–0.004 mg/kg SC, gd 6–15
Embryotoxicity						
Rat					+	(*177*)[g]
Chick					+	(*177*)[g]
Behavioral toxicity						
Rat					+	(*178*); repeated doses of 0.005 mg/kg SC at varying periods of pregnancy
Reproductive effects						
Sheep (1 generation, female)					–	(*111*); accidental exposure
Rat (3 generations)			–	(*179*); inhalation exposure, 0.001 or 0.0001 mg/m^3 for 10 months	–	(*173*);0.00025–0.004 mg/kg SC, gd 6–15
Rat (dominant lethal)				(*179*); inhalation exposure, 0.001 or 0.0001 mg/m^3 for 10 months	–	(*173*);0.00025–0.004 mg/kg SC, gd 6–15
Subchronic effects						
Rat (male and female, 90 days)	–	(*140,141*); tested at 0.1125, 0.05625, and 0.02813 mg/kg/day IP without atropine	–	(*149*), sarin I; (*150*) sarin II[h]	–	(*173*); 0.00025–0.004 mg/kg SC 5 days/week for 13 weeks

Abbreviations: gd, gestation day; SC, subcutaneous; IP, intraperitoneal.
[a]W+, weakly positive.
[b]Tested with and without activation.
[c]With stabilizers; type I, tributylamine; type II, diisopropylcarbodiimide.
[d]Tested at least three times at 2.4×10^{-3}, added as 20% of culture medium. GB I and II inhibited unscheduled DNA synthesis, indicating GB may inhibit DNA repair and allow permanent encoding of mutations, or it may protect DNA from damage.
[e]GA given SC 0.125, 0.05625, and 0.02813 mg/kg/day for gd 6–19 in rabbits.
[f]Type I and II tested by oral administration at 0.1–0.38 mg/kg/day for gd 6–15 in rats and at 0.005–0.015 mg/kg/day for gd 6–19 in rabbits.
[g]Pregnant rat received single 0.01 mg/kg SC dose or repeated 0.005 mg/kg SC doses, yielding reduced body weight of pups. Embryolethality observed in chicken eggs injected with 0.032 mg/egg; embryotoxicity observed at 0.03 mg/rat *in vitro*.
[h]CD rats given sarin I at 0. 0.075, 0.15, or 0.3 mg/kg/day (0.3 mg/kg/day is maximum tolerated dose) 5 days/week for 90 days by gavage. Sarin II was tested using the same design.

or DNA repair as indicated by UDS (*175*). Like GA, GB actually inhibited UDS (*175*). It is not known whether this inhibition reflects an ability of G-agent metabolites to scavenge free radicals and thus reduce DNA damage resulting in decreased need for repair. Alternatively, DNA repair capacity may be blocked by the agents with the result that permanent mutations could be produced (*180*).

Teratogenicity and reproductive toxicity. Tests for teratogenic effects of GB in the rabbit and rat were negative (*176*) (Table 6). Agent GB as sarin I and sarin II was tested via oral exposure in pregnant New Zealand White rabbits and CD rats. The number and status of fetal implants, individual fetal weights, and fetal malformations were evaluated; no evidence of developmental toxicity in the first 20 days of pregnancy was seen in either species, even at doses of GB that resulted in maternal toxicity or mortality.

Definitive tests of the effects of GB on reproduction have not been performed, but some data are available from a chronic exposure study of low levels of GB in rats. Denk (*179*) [as reviewed by Weimer et al. (*169*)] reported that no dominant lethal mutations or adverse effects on reproductive performance occurred in rats through three generations after exposure for 10 months to airborne GB at concentrations of 0.001 or 0.0001 mg/m^3 (see Table 6). These levels were so low that no overt signs of toxicity were produced. Another study evaluated testicular atrophy in Fischer rats after a 6-month exposure via SC or intraperitoneal injections of low doses of GB; no differences were found between treated and nontreated animals (*181*). Weimer et al. (*169*) reported no reproductive effects of long-term GB inhalation on any group of mice or rats except for the Fischer rats, which exhibited an increased incidence of testicular atrophy, a condition to which this strain is genetically susceptible. It was noted that this group of Fischer rats had undergone heat stress during the experiment. A follow-up study of unstressed Fischer rats exposed to the same concentrations (0, 0.01, and 0.0001 mg/m^3) for 12 or 24 weeks showed no testicular atrophy. The investigators concluded that the increase in testicular atrophy in the first experiment was due solely to heat stress during several weeks of the exposure period.

Agent VX

Chronic and subchronic toxicity. Goldman, et al. (*173*) reported the results of exposing male and female Sprague-Dawley rats to VX (0.00025, 0.001, or 0.004 mg/kg, SC) daily, 5 days/week, for 30, 60 and 90 days. Blood was assayed for RBC-ChE and plasma ChE activity, and a standard battery of clinical chemistry tests was performed. At 60 and 90 days, creatinine phosphokinase activity, an index of muscular injury, was also determined. Urine was collected for analysis during weeks 8 and 12 of the study. Body and organ weights were recorded and histopathological examination was performed on tissues. Hematological parameters were observed in a separate group of male and female rats exposed identically to VX in the first generation of a three-generation reproduction study (*173*).

RBC-ChE activity was significantly depressed in male and female rats at all VX doses for 30, 60, and 90 days. Plasma ChE was significantly depressed in both genders of rats given 0.001 mg/kg VX for 30 days and in both genders of the high-dose group at all exposure periods.

A slight decrease in body weight was observed in the high-dose group, but no consistent effects on organ weights were seen that could be ascribed to VX exposure. No dose-related changes were reported in clinical chemistry or urinalysis parameters. No histopathologic lesions were reported. Overall, the authors concluded that VX exposure sufficient to significantly depress RBC-ChE activity produced no subchronic toxic effects. No studies of long-term (chronic) VX exposure have been reported.

Carcinogenicity. As with the other nerve agents, the majority of animal studies on VX have dealt with the acute toxicity of this chemical agent. We have found no reports of carcinogenesis studies with VX. McNamara et al. (*37*) reported that there was no association of increased cancer in personnel working daily with VX; however, as with the other nerve agents, definitive studies on the carcinogenic potential of VX are lacking.

Genotoxicity. The potential of VX to cause genetic effects has been addressed in several studies supported by the U.S. Army Medical Bioengineering Research and Development Laboratory (*172,173*) (see Table 6). These studies include mutagenicity in bacteria (*S. typhimurium,* Ames assay), yeast (*Saccharomyces cerevisiae*), fruitflies (*Drosophila melanogaster*), and in a mammalian cell line (mouse lymphoma L5178Y). In the bacteria and yeast studies, VX was tested with and without metabolic enzyme activation to determine if VX metabolites might be mutagenic. The range of concentrations in the Ames assay included concentrations (1.093 mg/plate) that corresponded to approximately 40,000 times the estimated IV LD_{50} for humans (*172*). Results were negative in both the Ames assay (*172,173*) and the *S. cerevisiae* assays (*173*). In the *Drosophila* sex-linked, recessive lethal mutation test, only one mutation was observed at the higher VX concentration (0.004 mg/m^3), for a mutation percentage of 0.5% (*172*). A repeat test at the same concentration yielded no mutations. Thus, results were also negative for VX in this mutagenicity assay.

The fourth assay for mutagenic activity involved the use of mouse lymphoma cells, which may provide better health risk estimates for humans than tests using bacteria or yeast. Again, in this assay, VX was tested with and without metabolic activation. At lower concentrations (0.001–0.02 mg/ml), there was no statistically significant increase in the mutation frequency; at the higher test concentrations (0.02–0.1 mg/ml), there was a small but statistically significant increase in the number of mutants that appeared to be related to dose but not to activation (*173*). Compared with controls, this increase in mutations was less than the twofold increase set as a criterion for a positive mutagen (*182,183*); thus VX was considered by the authors to be a nonmutagen. Agent VX also gave negative results in the SCE assay, which tests for chromosomal damage rather than mutations.

Teratogenicity. Data on the potential of VX to affect fetal development (teratogenesis) come from the accidental exposure of sheep to lethal concentrations of VX and from controlled studies in rats and rabbits (see Table 6). Van Kampen et al. (*111*) reported studies on 79 surviving ewes in an accidental 1968 VX exposure in Skull Valley, Utah, in which 4500 of the 6300 affected sheep died or were killed. The dose that the exposed pregnant ewes received is not known, and the dose given another group of purposely exposed pregnant ewes is classified, making it difficult to evaluate this study. The accidentally exposed animals demonstrated clinical signs of toxicity; their RBC-ChE activities were depressed for up to 4 months after the initial intoxication, suggesting significant VX exposure. Under the conditions of both accidental and intentional exposure, no evidence of any significant developmental effects were noted in the offspring of the ewes.

The teratogenic potential of VX in rats and rabbits was tested by SC injection of 0.00025 to 0.004 mg/kg/day on gestational days 6 through 15 in rats and days 6 through 19 in rabbits. The pregnant animals were killed on day 20; the fetuses were removed and examined for body weight and for skeletal and organ abnormalities. Results of the studies in rats showed no statistically significant relationship between the dose of VX and any of the parameters studied (*173*). Results of the teratogenic studies in rabbits were also negative.

A preliminary study (*177*) suggested embryotoxicity of VX to rat fetuses after 0.03 mg SC doses to the mother and embryolethality to chick embryos at 0.032 mg/egg. Another preliminary study (*178*) suggested that VX may exert behavioral toxicity effects on the rat fetus after repeated SC doses of 0.005 mg/kg at varying times during fetal development. These results indicate that further study may be warranted.

Reproductive toxicity. Information to date suggests that neither acute nor chronic VX exposure has deleterious effects on reproductive potential. One data set comes from the 1968 Skull Valley incident of acute accidental exposure of sheep to unknown levels of VX (*111*). The exposed ewes were evaluated for their reproductive capacity by breeding them 5–6 months after exposure. Although the dose of VX received by the ewes is unknown, it was sufficient to cause signs of acute toxicity. No effects on reproductive capacity were found in these animals. The results of a three-generation assay for reproductive potential were reported to be negative in Sprague-Dawley rats (*171*) (Table 6). The doses of VX (0.00025–0.004 mg/kg, SC) were administered for 5 days per week for 21–25 weeks in the F_0 generation and for 24–27 weeks in the F_1 generation.

Implications for Public Protection

Several aspects of nerve agent physicochemical characteristics and toxicity have ramifications of particular importance in planning for public protection during continued storage and the active phase of the Chemical Stockpile Disposal Program. In view of its volatility, GB mainly constitutes an inhalation hazard. It would be expected to disperse more widely than the more dense and less volatile VX. However, all nerve agents are readily adsorbed into porous media such as wood, masonry, plastic, painted surfaces, and fabrics, from which they may outgas over varying periods of time (*98*). Thus, all nerve agents, but especially VX, may persist in common building materials and on agricultural crops, posing dermal and inhalation exposure hazards (*18*). Nondestructive decontamination methods are currently unavailable for porous surfaces and materials (*184*).

Agent control limits that are protective in terms of chronic inhalation exposure of both public and occupational populations are presented in Table 7 (*185*). Work is underway to develop equivalent control limits for drinking water and food items; currently, no such limits exist for porous surfaces (*186*). Existing technology is adequate to monitor low air concentrations to ensure compliance with atmospheric control limits, but further development is needed to detect comparably low levels in water and foodstuffs.

Even mild to moderate nerve agent exposures may produce mental and emotional effects that, together with effects such as nausea, may have a pronounced impact on public response to emergency warnings. The implications for an exposed civilian population include possible confusion, inability to follow directions provided in conjunction with public warnings from emergency personnel, inability or enhanced unwillingness to cooperate with authorities in the event of an evacuation notice (e.g., inability to drive), and limited ability to cooperate during decontamination and treatment procedures. Thus, early warning systems are of singular importance, as well as prior education of the public in adequate response measures.

Likewise, even mild or moderate exposures to emergency responders may render them mentally unable to return to duty for an extended time after decontamination and treatment result in the cessation of physical symptoms (*29,71*). In any case, responders should not return to any duty for which there is potential for reexposure to an anti-ChE agent before RBC-ChE activity returns to 80% or more of individual baseline nor before having been asymptomatic for at least 7 days (*187*). Thus, self-contamination in the course of emergency response activities such as casualty decontamination and treatment must be stringently avoided.

Conclusions

The overreaching concern with regard to nerve agent exposure is the extraordinarily high acute toxicity of these substances. These agents were designed to produce rapid incapacitation or death at exceedingly low doses. Inability to perform complex tasks or tasks requiring good vision (especially night vision) can result from low to moderate nerve agent doses. Such incapacitation may be a consequence of psychological effects alone or in combination with gastrointestinal, ocular, or respiratory effects and could have a significant negative impact on a population's ability to respond to emergency warnings and instructions.

The congressional mandate to destroy U. S. stockpiles of unitary chemical warfare agents by the end of this decade was the stimulus for gathering and analyzing the widely scattered literature on the toxicity of the stockpiled warfare agents as summarized here and, to some extent, in other reviews. The U. S. Army Chemical Stockpile Disposal Program (CSDP) is currently designed to carry out on-site, high-temperature incineration of organophosphate nerve agents stored in bulk or incorporated into munitions. The potential for an inadvertent release with off-site consequences is considered exceedingly small (probabilities of 10^{-4} to 10^{-10} for individual incidents) during continued storage or on-site stockpile destruction. The continued storage option is estimated to entail greater risks of fatalities than on-site disposal. The potential for low-probability but high-consequence releases has raised public concern in the vicinity of the stockpile sites and is resulting in an extensive upgrading of emergency preparedness in the civilian sector in advance of the CSDP. This analysis was prepared to assist the medical and emergency planning communities and to address various issues emerging as concerns in the course of public participation in the planning process.

The nerve agents of primary concern in the unitary stockpile are GB (sarin) and VX; GA (tabun) is present in a small quantity at only one location in the United States (Tooele Army Depot in Utah). Other states hosting or immediately adjacent to sites where nerve agents are stored include Alabama, Arkansas, Illinois, Indiana, Kentucky, Oregon, and Washington.

Marked differences in the volatility of the nerve agents result in significant differences in the potential geographic area affected by an inadvertent release, in persistence in the environment, and in the likely route of human exposure. Agent VX, being less volatile than GA and GB, would be expected to disperse less widely but to be more persistent and to present more of a contact hazard and potential ingestion hazard from contaminated agricultural products. Degassing of VX from porous surfaces (e.g., building materials) may pose problems of detection and decontamination before reentry can be determined to be safe. Agents GA and GB are primarily inhalation hazards, tend to disperse rapidly, and present little contact or ingestion hazard.

Agents GB and VX are both present at five sites (Table 1), raising concerns about potential interactions in the highly unlikely event that a combined release might occur. Limited experimental work with GB and VX plus evidence from OP insecticide research indicates potential for either synergism or antagonism, depending on

Table 7. Atmospheric control limits recommended by the Department of Health and Human Services for nerve agents (*185*)

Agent	General population (72-hr TWA), mg/m^3	Workplace (8-hr TWA), mg/m^3)
GA, GB	3×10^{-6}	1×10^{-4}
VX	3×10^{-6}	1×10^{-5}

TWA, time-weighted average.

whether exposure is simultaneous or sequential, and if sequential, on the order of exposure.

Organophosphate nerve agents are highly toxic by all routes of exposure. Their generally accepted principal toxic mechanism is the inhibition of acetylcholinesterase, although other mechanisms of action also appear to contribute to their toxicity. For example, experimental evidence implicates a role for excitatory amino acid receptors in nerve agent-induced convulsions and brain damage. Significant individual variations in sensitivity exist, probably due in part to variations in levels of endogenous RBC-ChE and other circulating cholinesterases as well as body fat depots. The estimates of human toxicity indices presented in Table 2 are based partially on animal data from rodent species that have protective enzymes such as carboxylesterase which humans lack. Thus, the estimates must be viewed with caution as humans may be more sensitive than some of the species on which LD_{50} values and other toxicity indices are based. Where human data were used in combination with animal data, they were based on experiments with young, healthy, adult male military volunteers. These volunteers are clearly not representative of the general population. Special populations, such as infants, may be more sensitive.

Tests of agent VX summarized in Table 5 demonstrate no potential to induce delayed neuropathy in any species. Agents GA and, to a somewhat greater extent, GB, have limited potential to induce OPIDN, based on studies in antidote-protected chickens exposed to supralethal doses. From these studies, it appears that OPIDN induction would be possible only at GA or GB doses that would be lethal to unprotected humans. One as-yet unverified small low-dose inhalation study in mice resulted in NTE inhibition, signs of ataxia, and histologically demonstrable axonal damage suggestive of OPIDN induction. No human exposures, experimental or accidental, have resulted in OPIDN induction.

Moderate or greater human exposures to GB have been associated in some individuals with transient difficulty in concentration, anxiety, and depression for days or weeks after exposure. Occupational exposures have been associated with subtle EEG changes of undefined significance. Animal studies suggest that cardiac toxicity may be associated with severe acute nerve agent exposure, but no conclusive evidence for effects have been observed in humans.

Although definitive lifetime carcinogenicity bioassay experiments have not been conducted with the nerve agents, no indication of carcinogenicity has been obtained from long-term low-dose studies of GB in several species. Genotoxicity screening tests were negative for GB and VX, while GA studies indicated only weak genotoxic potential in certain *in vitro* assays. That these compounds show little or no genotoxicity suggests that they are unlikely to be carcinogenic. Evidence to date suggests that the nerve agents are not teratogenic, nor do they have deleterious effects on reproductive function.

The rapid action of the nerve agents requires immediate decontamination and initiation of treatment, especially in cases of severe exposure. In some cases, continuous monitoring and repeated administration of antidotes as symptoms indicate may also be needed. In severe exposures, other supportive measures such as artificial ventilation will be necessary. Multiple cases of nerve agent poisoning could severely tax local medical capabilities, especially given the need for simultaneous decontamination and treatment by personnel wearing protective clothing and using somewhat cumbersome procedures to avoid self-contamination. A remote (probability ranging from 1 in 10^{-4} 1 to 1 in 10^{-10}) possibility exists of inadvertent off-site agent contamination during storage or the CSDP. A possibility also exists for civilian exposure resulting from accidental disturbance of nonstockpile sites or OP agent use by terrorists. Such contingencies require that both first responders and other medical personnel be well versed in the range of acute toxic manifestations of nerve agent exposure and appropriate treatment procedures.

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